Effect of Anticholinergic Agents on the Intestinal Absorption of ^{59}Fe Ferrous Citrate

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The absorption of ^{59}Fe ferrous citrate has been studied in humans after administration of the anticholinergic agent, hexocyclium methosulfate, and in rats, after atropine. In both species, anticholinergics produced a decrease in absorption. The effect of atropine on iron absorption persisted when the radioiron was delivered intragastrically, mixed with gastric juice, but not when administered intraduodenally. Another set of experiments showed that iron absorption decreases when radioiron is intraduodenally delivered in deproteinized acid gastric juice, or in 0.10 N HCl, but not when mixed with neutralized gastric juice. It is concluded that anticholinergic drugs inhibit iron absorption by an effect on a gastric factor necessary for optimal iron absorption, and not through a decrease in acid secretion.

Despite initial conflicting opinions (1–5), most of the recent experimental and clinical evidence, obtained by the use of more reliable technics, suggests that the stomach plays an important role in iron absorption (6–16). A consequence of partial gastrectomy in man is malabsorption of food iron (17–20), and vagotomy is not entirely free from hematologic consequences, as shown by lower hemoglobin and serum iron values (21, 22). It was considered of interest to study whether the administration of anticholinergic drugs which inhibit gastric secretion also interferes with iron absorption. In this paper, we discuss experiments designed to examine the absorption of radioiron (^{59}Fe ferrous citrate) in humans and in rats receiving anticholinergic drugs.

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MATERIAL AND METHODS

Instrumentation

Whole body counting was done with a 20 × 10 cm Harshaw NaI thalium-activated crystal. This was housed in a 5-cm thick lead container provided with a funnel-shaped collimator located around the crystal's open end. This semicone was 30 cm high and 45 cm wide in its inferior diameter. Its thickness was tapered from 3.7 cm at the crystal end to 0.5 cm at the distal end. The electronic equipment consisted of a 400 channel TMC analyzer, set at 4 keV per channel. Average background at the photopeak was 60 counts/min and the efficiency was approximately 450 counts/min/μCi. The counting time of 10 minutes allowed accumulation of sufficient counts for a minimum accuracy of ±2% in all measurements.

Protocol

Experiments in humans. Six male physicians were investigated. The experimental nature of the

study was fully explained to them. The subjects were normal and none was anemic. The radioiron used in the tests was ^{59}Fe ferrous citrate* (specific activity 26.0 mCi/mg Fe), 10 μCi of which were dissolved in water containing 250 μg of ferrous sulfate (92.4 μg Fe^{++}). This mixture and the rinsings of the beaker were ingested after an overnight fast. Whole body counts were performed 4 hours later. This value served as the 100% reference point of the administered dose (23). The crystal was placed at a distance of 1.7 m from a stretcher on which the subject reclined. It was centered on the xyphoid, and its isosensitivity arc included the head and knees of the subject. The difference in sensitivity between the anterior and posterior surfaces of the body amounted to 10%.

Sixty minutes before administration of radioiron, the subjects received a dose of 62.5 mg of hexocyclium methosulfate (Tral),* an anticholinergic agent. The dose was repeated 6 hours later. In all of the subjects, this dose produced the usual dryness of the mouth secondary to the use of a rather high dose of a parasympatholytic agent, but no other symptoms were observed. Four hours after ingestion of the radioiron, whole body counting was done. Every 5 days thereafter, for 15 days, a plateau of body radioactivity was reached in all subjects. After correcting for the physical decay of the isotope, the percentage absorbed was calculated from the ratio of the final to initial counts.

When no radioactivity was detected in the body, the same subjects again received the same dose of radioiron, this time without the anticholinergic agent, and the same procedure was followed. Each person thus served as his own control.

Experiments in rats. Male Sprague-Dawley rats, weighing approximately 250 g and fed a normal laboratory diet, were used. All experiments were started after an overnight fast. Iron absorption was measured after administration of ^{59}Fe ferrous citrate, without carrier, in two different doses: When the radioiron was given directly, intragastric 0.5 μCi was used, with 0.25 μg being the total amount of iron per dose. When given with gastric juice, 20 μCi of radioiron were mixed with 5 ml of gastric secretion, and 0.5 ml of the mixture was administered. The total amount of iron per dose was 0.20 μg. In the experiments in which the isotope, alone or in a mixture with gastric juice, was given intragastrically, it was administered through an indwelling polyethylene tube. When the radioiron was given intraduodenally, a small laparotomy was performed under light ether anesthesia, and the mixture of gastric juice and radioiron was injected through a needle across the stomach and the pylorus into the first portion of the duodenum. The incision of the abdominal wall was then closed.

In every experiment, radioactivity was measured as follows: One hour after administration of the isotope, the total body radioactivity was measured. This was done by placing the animal in a tight-fitting container in order to avoid counting rate variability caused by changes in body position. The distance to the crystal was 60 cm, and its isosensitivity area included the entire animal. Total body counting of the isotope was performed daily for 7 days. This period was enough for the unabsorbed part of the dose to be fully excreted, and a plateau of radioactivity was attained. The percentage of radioiron absorbed was then calculated as in humans.

In rats, the anticholinergic used was atropine sulfate, subcutaneously administered in two doses of 1 mg with an interval of 6 hours. No obvious toxic symptoms were observed with this dose. The radioiron was administered 1 hour after the first dose of atropine. This dose was chosen after experiments in which the pylorus was ligated in 10 rats, and gastric secretion collected according to the procedure of Shay (24). The rats were killed 4 hours after ligation of the pylorus. The gastric contents were analyzed as to volume and hydrochloric acid concentration in milliequivalents per liter. Five of these rats had received 1 mg of atropine sulfate subcutaneously 1 hour before the operation. The volume of the gastric contents in the controls was 6.15 ± 0.46 ml; it decreased to 1.10 ± 0.30 ml in the rats receiving atropine. The concentration of hydrochloric acid fell from 62.30 ± 12.19 mEq/liter in the controls to 0.031 ± 0.01 mEq/liter in the rats with atropine.

When ^{59}Fe ferrous citrate was given mixed with gastric juice, the latter was collected after ligation of the pylorus in 3 rats for 3 hours. The rats were then killed and the gastric contents were pooled together. The pH of the pooled gastric secretion was always below 2. Then 20 μCi of radioiron were added for each 5 ml of gastric juice. This mixture was further incubated in a water bath at 37 C for 1 hour.

In the experiments in which neutralized gastric juice was used, the radioiron was mixed as above, then the mixture was neutralized by the slow addition of 12.5 M NaOH through a fine capillary

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tube under constant agitation. pH measurements were made with a Radiometer titrator.

When deproteinized gastric juice was used, the gastric contents were treated with 10% trichloroacetic acid and the filtrate, after precipitation, was mixed with 1.5 volume of acetone and heated at 40 C for 1 hour. With this procedure, a precipitate is formed which contains all the iron-binding substances present in gastric juice (10). Then 20 μCi of ^{59}Fe ferrous citrate was added to each 5 ml of the acetone-free filtrate.

Statistical Analysis

Statistical evaluation was performed with the use of the Student's t test. As used in the text, *significant* means $P < 0.05$, and values are means ± SE.

RESULTS

Experiments in Humans

Table 1 shows that after administration of hexocyclium methosulfate, there is an important decrease in the percentage of radioiron absorbed in all subjects; in some of them, absorption was completely inhibited by the anticholinergic.

Experiments in Rats

When radioiron was delivered intragastrically, absorption decreased significantly in rats receiving atropine, compared to the controls (Table 2). In order to test if this effect of atropine is secondary to the decrease in acidity induced by the drug, ^{59}Fe ferrous citrate was given after mixing and incubation in acid gastric juice. In this condition, there was a significant diminution of radioiron absorption with respect to the animals receiving radioiron without previous mixing and incubation in gastric juice. Notwithstanding, the administration of atropine induced a further decrease in absorption. The inhibition resulting from atropine administration was not dependent on an increase in gastric pH, since both mixtures came from a pool of gastric secretion with a pH of 1.5 (Table 2).

Table 1. Effects of Hexocyclium Methosulfate (Tral) on ^{59}Fe Ferrous Citrate Absorption in Humans

	Percentage of $^{59}Fe^{++}$ absorbed	
Subject	Control period	With Tral
1	33.1	0
2	7.2	0.4
3	11.3	1.7
4	10.4	1.9
5	7.1	1.3
6	13.7	8.6

To test the possibility that atropine inhibition of Fe++ absorption would be due to an intestinal effect of the drug and not to its action on gastric secretion, radioiron mixed with gastric juice, as in the preceding experiment, was administered to rats, in a volume of 0.5, directly into the first portion of the duodenum. ^{59}Fe++ absorption under these conditions was of the same order of magnitude as when radioiron mixed with gastric juice was placed in the stomach (Table 3). When the experiment was repeated in animals receiving atropine, the percentage absorbed was not statistically different from that absorbed in animals without atropine.

When gastric juice was treated with trichloroacetic acid and acetone to precipitate proteins and all iron-binding substances present, and only then mixed with radioiron, and injected into the duodenum, there was a significant decrease in iron absorption with respect to the amount absorbed when radioiron mixed with whole gastric juice was injected.

There was also a decrease in ferrous citrate absorption when the isotope was injected into the duodenum in an artificial solution of 0.10 N hydrochloric acid. On the contrary, when the isotope was mixed with gastric juice, and the mixture neutralized to pH 7.0 and then injected into the duodenum, the absorption was not signifi-

cantly different from that found when the radioiron mixed with gastric juice at pH 1.8 was injected into the duodenum (Table 3).

DISCUSSION

Our results clearly indicate that anticholinergic drugs decrease the absorption of ^{59}Fe ferrous citrate in the human and the rat. Since atropine had no effect on radioiron absorption when the isotope is delivered directly into the duodenum, this effect of the drug must be secondary to a change in gastric function. The fact that malabsorption of the isotope persists when the radioiron is given mixed with gastric juice at a low pH shows that the cause of the defect is more complex than the mere decrease in gastric acidity induced by the drug.

Table 2. Effect of Atropine Sulfate on ^{59}Fe Ferrous Citrate Absorption in Rats After Intragastric Administration of the Isotope

	Percentage of $^{59}Fe^{++}$ absorbed				
Form of administration	No. of experiments	Controls	No. of experiments	With atropine	*P*
^{59}Fe ferrous citrate	15	42.42 ± 5.85	11	10.77 ± 3.20	<0.001
^{59}Fe ferrous citrate mixed with gastric juice and incubated	6	15.25 ± 1.52 *P* < 0.001	6	7.28 ± 1.61 *P* > 0.10	<0.001

Numbers are means ± SE.

Table 3. ^{59}Fe Ferrous Citrate Absorption in Rats After Intraduodenal Administration

	Percentage of $^{59}Fe^{++}$ absorbed				
Form of administration	No. of experiment	Controls	No. of experiment	With atropine	*P*
^{59}Fe ferrous citrate mixed with gastric juice	6	17.41 ± 1.75	6	14.61 ± 2.62	>0.90
^{59}Fe ferrous citrate mixed with neutralized gastric juice	8	14.84 ± 2.94	*P* > 0.90		
^{59}Fe ferrous citrate mixed with deproteinized gastric juice	5	8.04 ± 2.39	*P* < 0.005		
^{59}Fe ferrous citrate mixed with 0.10 N HCl	7	11.48 ± 2.63	*P* < 0.05		

Numbers are means ± SE.
The differences (*P*) of the last three forms of administration are compared with those of the first.

As iron absorption varies according to the form in which the metal is ingested, and according to the composition of the diet (25), and since food iron is poorly absorbed in comparison to ferrous salts (27, 28), observations related to one form of iron cannot be applied to another (26). In our experiments, we used the ferrous form of iron. Ferrous salts are more easily ionized and more available to absorption than are the ferric forms (31). The citrate salt we used might also improve absorption, as citrate forms complexes with iron that keep it in solution even in neutral and weak alkaline solutions (29, 30). Nevertheless, if a defect is shown with a very small dose of a well absorbed form of iron, the defect is likely to persist with a high intake of a less soluble or absorbable form, although the magnitude of the defect may be different (6).

Our results do not permit us to determine the exact mechanism of this action of anticholinergics, but they point to a gastric effect independent of the one on acid secretion. It has been postulated that there is an active compound in gastric juice which forms complexes with inorganic iron to render it available to the intestinal epithelial cells (8, 10, 34). The secretion of this compound could be controlled by a cholinergic mechanism and might diminish in gastric juice after administration of atropine. The existence of this active compound is compatible with the decreased absorption observed in our experiments when $^{59}Fe^{++}$, mixed with deproteinized gastric juice, or in 0.10 N hydrochloric acid was given intraduodenally. The same explanation could account for the fact that radioiron mixed with gastric juice and then neutralized is as well absorbed when given intraduodenally as when given mixed with normal acid gastric juice. All these experiments could be explained by a protein or peptide binder necessary for iron absorption. This binder is not present in artificial hydrochloric acid and disappears after protein precipitation. On the other hand, when iron is added to gastric juice, and the mixture is then neutralized, iron would be kept in solution by the complex.

It is still difficult to explain why atropine produces a decrease in radioiron absorption when the isotope is given intragastrically mixed with normal gastric juice. In this respect, it is of interest that we also observed a striking decrease in $^{59}Fe^{++}$ absorption without atropine. This decrease occurred when the isotope was given intragastrically after being mixed with gastric juice collected during a 3-hour period when the pylorus was ligated, and was then further incubated for 1 hour. This could be due to a change in the nature of gastric contents. Duthie et al (7) showed that prolonged incubation of radioiron with intestinal fluid changed its behavior and made iron no longer suitable for absorption. In the stomach, something of this kind could also happen. This could be due either to changes in an iron-binder necessary for optimal absorption, or to a reaction between the iron and a factor secreted or produced by digestion that limits its absorption. Anticholinergic drugs increase gastric emptying time. In the rats receiving $^{59}Fe^{++}$ intragastrically mixed with normal gastric juice, atropine could result in a more prolonged action of pepsin and acid on components of gastric secretion. This could result either in the disappearance of a substance necessary for iron absorption, or in the appearance of another that interferes with absorption.

If the defect in ferrous citrate absorption induced by anticholinergic drugs is extended to other forms of dietary iron, it could explain the hematologic alterations that sometimes are seen after vagotomy (21, 22).

It could also be of importance in patients who receive such drugs for very long periods, especially in the case of patients with peptic ulcer who have experienced recent bleeding and anemia.

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