Pharmacology Biochemistry & Behavior, Vol. 11, pp. 331–334. Printed in the U.S.A.

Coeliactomy: Differential Toxiphobia Conditioning with Apomorphine and Copper Sulfate[1]

JAMES R. MARTIN

Institute for Behavioral Science, Swiss Federal Institute of Technology, Zürich, Switzerland 8092

Received 18 April 1979

MARTIN, J. R. *Coeliactomy: Differential toxiphobia conditioning with apomorphine and copper sulfate.* PHARMAC. BIOCHEM. BEHAV. 11(3) 331–334, 1979.—Thirsty coeliac ganglionectomized and sham operated rats consumed more of a novel fluid after a series of presentations, each followed by saline injection, than when apomorphine was injected or copper sulfate intragastrically intubated. Sham rats drank significantly more of this novel solution than ganglionectomized rats following treatments with a very low dose of the peripherally-acting emetic copper sulfate that locally irritates the gastric mucosa. However, sham and ganglionectomized rats drank comparable amounts of the novel fluid following treatments with saline and the two groups drank comparable amounts following treatments with the centrally-acting emetic apomorphine. The results suggest that coeliac ganglionectomy eliminates some, but not all, of the afferent nerves transmitting information concerning visceral toxicosis (produced with intragastric copper sulfate) to the brain.

Conditioned toxiphobia Taste aversion learning Coeliac ganglionectomy Afferent nerves Copper sulfate Apomorphine Gastric irritation

INVESTIGATION of the neuroanatomical basis of taste aversion conditioning has provided evidence for its similarity with that of the emetic system. Borison and Wang [2] postulated a model of the emetic mechanism that included sensory inputs via afferent visceral nerves and from the bulbar area postrema. This model was supported by the demonstration that trans-thoracic vagotomy increased the threshold dose of oral copper sulfate required to induce vomiting in dogs [9]. Although bilateral sympathectomy alone had no obvious effect on emesis, combined vagotomy and sympathectomy very greatly increased the emetic threshold for copper sulfate above that observed with vagotomy alone. Apomorphine-induced vomiting was blocked by ablation of the area postrema but not by peripheral nerve section, indicating the central locus of action of this drug. Further support for this model was provided by the demonstration that section of vagi or sympathetic nerves alone did not abolish peritonitis-induced vomiting in cats, but that the combined denervation procedure did [8].

At present the neural basis of afferent inputs to brain regions controlling toxiphobia conditioning is only partially delimited. Berger, Wise and Stein [1] demonstrated that destruction of the chemosensitive area postrema prevented toxiphobia learning in rats when methylscopolamine, but not d-amphetamine, was used as the toxic, unconditioned stimulus. Although Martin, Cheng and Novin [5] failed to observe any significant deficit in taste aversion conditioning in bilateral subdiaphragmatically vagotomized rats when lithium chloride was the toxic agent, subsequent research has noted that toxiphobia conditioning in vagotomized rats is impaired when either intragastric or intraperitoneal copper sulfate was used to induce toxicosis [3]. The results of these investigations suggest that the area postrema and the abdominal vagi can play an important role in taste aversion learning when certain substances are used to induce toxicosis. The present study evaluates the effect of coeliac ganglionectomy on taste aversion learning produced with the centrally-acting emetic apomorphine or a peripherally-acting treatment involving intragastric intubation of a low concentration of copper sulfate. The ganglionectomy procedure used has previously been reported to decrease the efficacy of hepatic-portal infusion of 2-deoxy-D-glucose in eliciting feeding, an effect ascribed to the destruction of afferent visceral nerves [7]. However, this surgical procedure had no longterm effects on spontaneous daily food and water intake [6].

METHOD

Animals

A total of 56 male Wistar-derived RLA/Verh rats, approximately 6 months of age at the time of surgery, were used. Throughout the experiment the rats were individually housed in Macrolon cages in animal quarters with a 12:12 hr light-dark cycle. Food (Nafag Lab Pellets, No. 890) and tap water were continuously available except when the experimental procedure specified otherwise.

[1]The assistance and advice provided by Professor K. Baettig and Professor D. Novin is gratefully acknowledged. Requests for reprints should be sent to: J. R. Martin, Institut für Verhaltenswissenschaft, ETH-Zentrum, Turnerstrasse 1, 8092 Zürich, Switzerland.

Procedure

All surgery was done under aseptic conditions in rats anesthetized with Equithesin. Thirty-two rats underwent coeliac ganglionectomy (Gangx group), which was accomplished by removing all visible nervous tissue, as well as the surrounding connective and adipose tissue, within the region delimited by the abdominal aorta and the coeliac and mesentaric arteries as described in Lambert [4]. Sham surgery was performed on 24 rats (Sham group) by manipulating the visceral organs, but without extirpation or sectioning. Following surgery, all rats received penicillin (50,000 units, IM).

Behavioral testing began 5–7 wk postsurgery. The subjects were habituated to a schedule of 12 hr water deprivation for 7 days. Water bottles were removed at light offset and returned 12 hr later at light onset for a 1 hr period of access. Then, the water bottles were again removed, for a 2–3 hr period, before being returned for the remainder of the day. Following the week of habituation, the 50-day experiment began and consisted of 3 phases: final water-deprivation training (Days 1–3), toxiphobia acquisition phase (Days 4–34) and extinction phase (Days 35–50). Throughout the entire 50-day experiment, rats remained on the water deprivation regimen previously described. However, during the acquisition phase the subjects received a palatable solution of 0.1% sodium saccharin in 3% glucose (SG) during the 1-hr period of fluid access following the 12 hr deprivation period. SG was presented on every third day for 30 days and water on the other days. During this acquisition phase, presentation of SG was consistently followed by injection of physiological saline (IP or IG), apomorphine hydrochloride (5 mg/kg, IP), or copper (II) sulfate 5-hydrate (1 mg/kg, IG). Apomorphine and copper sulfate were freshly prepared in physiological saline and the doses refer to the salt. Injection volume was 2.0 ml/kg for intraperitoneal and 10 ml/kg for intragastric administration. The 29 Gangx rats that survived the surgical intervention and the 24 Sham rats were assigned to 6 groups: Sham-saline (N=8), Sham-apomorphine (N=8), Sham-copper sulfate (N=8), Gangx-saline (N=10), Gangx-apomorphine (N=9), and Gangx-copper sulfate (N=10). An extinction phase followed the toxiphobia acquisition phase. During the extinction phase the SG fluid was presented every day during the 1-hr test period, but no injection followed.

Intake data from the 1-hr tests at critical phases of the experiment were analyzed with either the Wilcoxon matched-pairs signed-ranks test or the Mann-Whitney U test. All comparisons were based on nondirectional p-values. Following the completion of behavioral testing the rats were sacrificed and an autopsy performed to evaluate the success of the denervation procedure.

RESULTS

Consumption of water or the SG solution in the 50 successive daily tests for coeliac ganglionectomized and control rats is shown in Fig. 1. The data for rats receiving saline via intraperitoneal and intragastric routes were not different and thus were averaged. Gangx and Sham rats treated with saline significantly increased (p's<0.01) SG intake during the acquisition phase (Day 4 vs 35), but exhibited no further increase during the extinction phase (Day 35 vs 50). In contrast, Gangx and Sham groups injected with apomorphine exhibited a nonsignificant reduction (p's<0.10) in SG intake during acquisition, but subsequently exhibited a significant

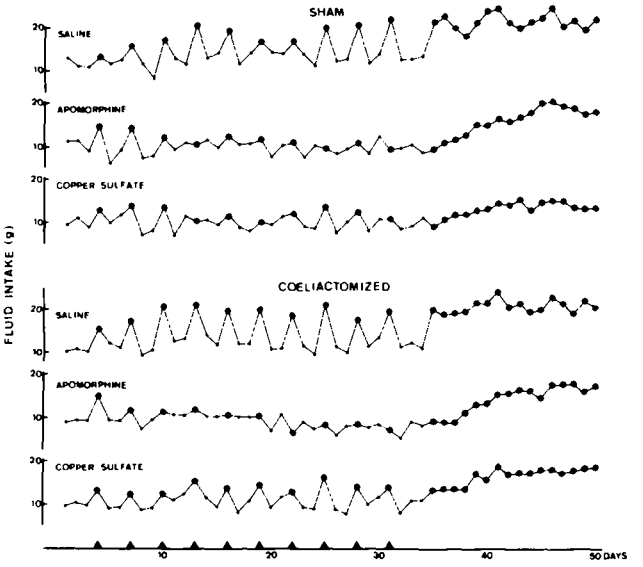

FIG. 1. Daily consumption of water or saccharin-glucose (SG) by fluid-deprived sham operated or coeliac ganglionectomized rats during a 1-hr test. Presentation of SG was followed by administration of saline, apomorphine, or copper sulfate during the acquisition phase. Triangles indicate occurrence of these injections and asterisks indicate tests when SG was presented.

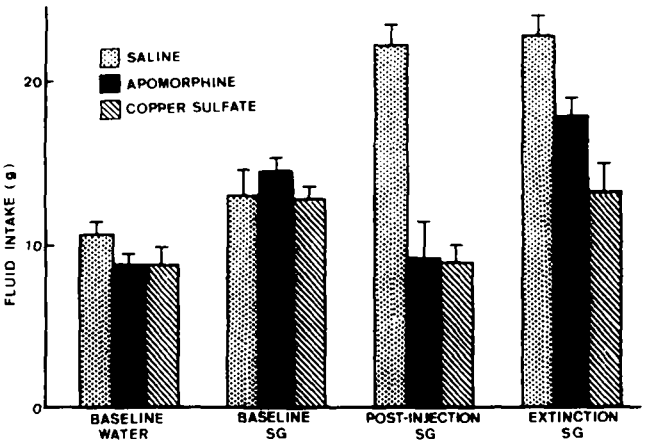

FIG. 2. One-hour water or saccharin-glucose (SG) consumption by fluid-deprived sham operated rats prior to drug treatment, at the end of the acquisition phase, and at the end of the extinction phase.

increase (p's<0.02) in SG consumption during extinction. Sham rats intubated with copper sulfate significantly decreased (p<0.01) their SG consumption during the acquisition phase, whereas Gangx rats did not. Both Gangx and Sham rats significantly increased (p's<0.05) SG intake when copper sulfate intubation no longer followed SG presentation (extinction).

Water and SG consumption at critical points of the experimental procedure for Sham rats treated with saline, apomorphine, or copper sulfate is shown in Fig. 2. Prior to drug treatment (Day 3), water intake for these three groups was comparable, as was SG consumption on first exposure (Day 4). Sham rats injected with saline drank significantly more SG at the end of the acquisition phase (Day 35) than those injected with either apomorphine (p<0.01) or copper

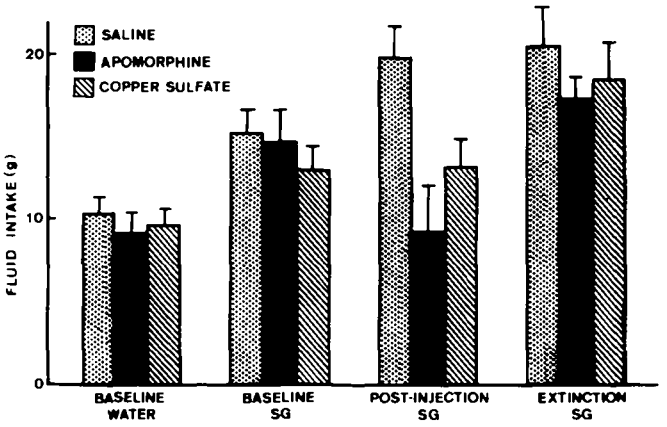

FIG. 3. One-hour water or saccharin-glucose (SG) consumption by fluid-deprived coeliac ganglionectomized rats prior to drug treatment, at the end of the acquisition phase, and at the end of the extinction phase.

sulfate ($p<0.01$). Figure 3 shows water and SG intake for Gangx rats treated with saline, apomorphine, or copper sulfate. Prior to drug treatment (Day 3), water intake was comparable for these three groups as was intake of the SG solution on the first day of exposure (Day 4). Gangx rats injected with saline drank significantly more SG in a test at the end of the acquisition phase (Day 35) than did the Gangx rats injected with apomorphine ($p<0.02$) or copper sulfate ($p<0.02$). Direct comparison of SG intake by Gangx and Sham rats for each injection condition indicated that although Gangx rats treated with copper sulfate drank significantly more of the SG solution at the end of the acquisition phase (Day 35) than did Sham rats treated with copper sulfate ($p<0.05$), intake at the conclusion of apomorphine or saline treatment was not different between Gangx and Sham groups.

The mean body weights (g) of the 6 experimental groups at the time of surgery were: 365 (Sham-saline), 362 (Sham-apomorphine), 360 (Sham-copper sulfate), 358 (Gangx-saline), 365 (Gangx-apomorphine), and 382 (Gangx-copper sulfate). At the beginning of the acquisition phase these group means were: 402, 396, 384, 408, 396, and 407, respectively. At the conclusion of the extinction phase these group means were: 407, 401, 381, 400, 399, and 403, respectively. An autopsy was performed on all rats at the conclusion of behavioral testing. Following sacrifice with an overdose of Equithesin, the region delimited by the abdominal aorta and the coeliac and mesenteric arteries was examined. Although neural tissue was not observed within this region in the Gangx rats, the presence of extensive adhesions prevents any definite conclusion as to whether neural tissue was completely excised during surgery. It should be noted that the surgical procedure used probably resulted in removal of the mesenteric ganglia, in addition to the destruction of the coeliac ganglia.

DISCUSSION

The present investigation provides further evidence that the neuroanatomical basis of afferent inputs to brain mechanisms controlling emesis and those involved in toxiphobia conditioning are similar. Earlier research has demonstrated that the bulbar emetic mechanism receives input from visceral afferent fibers nominally designated as parasympathetic and sympathetic nerves and from the bulbar area postrema. Wang and Borison [10] demonstrated that apomorphine acted directly on the area postrema to elicit vomiting since ablation of this structure rendered dogs refractory to the emetic effects of apomorphine. In contrast, combined vagotomy and sympathectomy did not alter the effectiveness of apomorphine in producing emesis. Oral administration of low doses of the peripherally-acting emetic copper sulfate elicited vomiting in area postrema-ablated dogs, but was relatively ineffective in those that received vagotomy alone. Bilateral destruction of the sympathetic ganglia from T_8 to the last sacral ganglion had no obvious effect on the threshold for copper sulfate-induced emesis, but combined vagotomy and sympathectomy resulted in a very substantial increase in refractoriness above that observed with vagotomy alone. The role of the sympathetic nerves has been reported to be equal to that of the vagi for emesis resulting from experimentally-induced peritonitis in cats. Neither low intrathoracic vagotomy nor bilateral sympathectomy-splanchnectomy alone abolished the vomiting response, but combined denervation did [8].

Experiments in rats have provided evidence that toxiphobia conditioning also involves sensory input via visceral afferent fibers and the area postrema. Berger *et al.* [1] noted that the integrity of the area postrema was necessary for toxiphobia conditioning to methylscopolamine. Martin *et al.* [5] demonstrated that bilateral abdominal vagotomy did not prevent toxiphobia conditioning when lithium chloride was used as the toxic agent, and argued that sensory input from the remaining visceral nerves and/or the area postrema probably mediated the taste aversion learning observed. Subsequently, it has been reported that bilateral subdiaphragmatic vagotomy prevented taste aversion learning when a novel taste was paired with intragastric or intraperitoneal copper sulfate injection in rats [3]. The results of the present study provide evidence that coeliac ganglionectomy impairs toxiphobia conditioning to intragastric copper sulfate but not to intraperitoneal apomorphine injection. Although the results of the study by Coil *et al.* suggest that afferent information concerning copper sulfate-

induced stomach irritation is transmitted predominantly by vagal fibers, it is difficult to evaluate the magnitude of the reported effect because only relative changes in consumption of the novel fluid were reported and it has previously been noted that baseline fluid intake by vagotomized rats is often minimal [5]. In addition, it has been noted that the minimal effective emetic dose of oral copper sulfate does not vary with body weight [10], therefore use of a dose adjusted for body weight in that study probably resulted in a lower dose for the vagotomized group since vagotomy typically results in a significant reduction in body weight [5]. Thus, the relative deficit in taste aversion learning following destruction of the coeliac gangli or transection of the vagi is not clear.

REFERENCES

1. Berger, B. D., C. D. Wise and L. Stein. Area postrema damage and bait shyness. *J. comp. physiol. Psychol.* **82:** 475–479, 1973.
2. Borison, H. L. and S. C. Wang. Physiology and pharmacology of vomiting. *Pharmac. Rev.* **5:** 193–230, 1953.
3. Coil, J. D., R. C. Rogers, J. Garcia and D. Novin. Conditioned taste aversions: Vagal and circulatory mediation of the toxic unconditioned stimulus. *Behav. Biol.* **24:** 509–519, 1978.
4. Lambert, R. *Surgery of the Digestive System in the Rat.* Springfield, IL: Charles C. Thomas, 1965, p. 474.
5. Martin, J. R., F. Y. Cheng and D. Novin. Acquisition of learned taste aversion following bilateral subdiaphragmatic vagotomy in rats. *Physiol. Behav.* **21:** 13–17, 1978.
6. Rezek, M., K. Schneider and D. Novin. Regulation of food intake after vagotomy, coeliactomy, and a combination of both procedures. *Physiol. Behav.* **15:** 517–522, 1975.
7. Schneider, K., M. Rezek and D. Novin. Effects of visceral sympathectomy on 2-deoxy-D-glucose induced eating. *Physiol. Behav.* **16:** 55–58, 1976.
8. Walton, F. E., R. M. Moore and E. A. Graham. The nerve pathways in the vomiting of peritonitis. *Archs Surg.* **22:** 829–837, 1931.
9. Wang, S. C. and H. L. Borison. Copper sulphate emesis: A study of afferent pathways from the gastrointestinal tract. *Am. J. Physiol.* **164:** 520–526, 1951.
10. Wang, S. C. and H. L. Borison. A new concept of organization of the central emetic mechanism: Recent studies on the sites of action of apomorphine, copper sulfate and cardiac glycosides. *Gastroenterology* **22:** 1–12, 1952.