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APOMORPHINE-INDUCED VOMITING IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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Abstract—1. The LD_{50} for a 7-day period following intraperitoneal injection of apomorphine HCl was calculated to be 158 mg/kg in rainbow trout.

2. Intraperitoneal injection of apomorphine at doses of 60 mg/kg or greater caused vomiting of plastic balls which had been placed in the stomachs of rainbow trout.

3. Apomorphine-induced effects included vomiting, vomiting behavior, toxicity, increased respiration, impaired motor control and equilibrium, and increased aggression.

4. The vomiting control mechanism of trout may be similar to that described in mammals.

INTRODUCTION

Vomiting, or emesis, has been well described in mammals but little has been published about its occurrence in other vertebrates. Indirect evidence suggests that large, predatory fish were vomiting over 300 million years ago. Partially disarticulated "prey" skeletons in Pennyslvanian black shale deposits (Zangerl and Richardson, 1963) have been interpreted as "vomite" (Wood, 1980) or fossilized vomit. Extant fish possess the basic brainstem architecture essential for vomiting control (Borison *et al.*, 1981), although this does not in itself indicate that fish will respond to emetic drugs.

Fish emesis is not often reported or studied, but it is often encountered in various situations by people handling and observing fish (Table 1). Apparently, the few studies done to date have emphasized development of a technique for collection of the stomach contents of live fish. According to the primary literature, arsenous acid (Markus, 1932; Jernejcik, 1969), antimony potassium tartrate or tartar emetic (Jernejcik, 1969; Foster, 1977) and apomorphine (Jernejcik, 1969) are the only emetics that have been used in fish. *Esox*, *Micropterus* and *Stizostedion*, used in the above studies, are the only fish that have been shown to respond to emetic drugs. We are unaware of (published) emetic studies in salmonids, although some researchers have noted the presence or absence of vomiting in trout during other types of studies (Table 1).

The purpose of this study was to examine the effects of the emetic apomorphine hydrochloride on rainbow trout. Apomorphine was chosen because it has been shown to act selectively through a chemoreceptor trigger zone in the medulla, to stimulate the brain center that controls vomiting in mammals (Wang and Borison, 1952). Our objectives were:

(1) to determine the LD_{50} and assess the toxicity of apomorphine in rainbow trout;

(2) to determine the dose response and time course for survival, vomiting and vomiting behavior; and

(3) to describe apomorphine-induced effects on respiration, motor control, aggressive behavior and gastric pH.

We found that intraperitoneal injection of apomorphine caused trout to vomit. This finding suggests that trout may possess a brain vomiting mechanism similar to that of mammals.

MATERIALS AND METHODS

LD_{50}

Treatment of experimental animals. The mortality caused by a single intraperitoneal injection of apomorphine–HCl during a 7-day period was investigated. Rainbow trout (*Salmo gairdneri*) ranging between 170 and 210 mm total length (TL) and 50-75 g were tested at a private hatchery. The fish were randomly assigned to groups ($n = 15$), and injected with the vehicle control or one of five drug dosages (20, 50, 100, 200, or 400 mg/kg). The animals were fin-clipped for identification, and combined in a screened bin at the end of a production raceway. Observations were made at least twice daily; dead fish were collected and the deaths recorded. At the end of 7 days, all remaining fish were recaptured and identified.

Drug preparation. Apomorphine–HCl (Sigma Chemical Co.) concentrations were individually prepared using a Teflon-on-glass tissue grinder and 0.5% Triton-X100 (Sigma) as a dispersing agent in distilled water (which had been further purified in two Barnstead ion exchange columns and passed through a Nanopure filter, 18.5 megohm-cm, pH 5.5). The two highest concentrations were green, milky suspensions; the other three concentrations were fully dissolved at the time of injection. Triton-X100 (0.5%) and purified distilled water was also used as a vehicle control. The dissolved drug was kept on ice in stoppered, foil-wrapped flasks during transport and used within 60 min.

Analysis of data. The LD_{50} value was calculated using the SAS (Allen, 1982) computer software probit analysis. Since one fish from each of three groups (control, 50 and 100 mg/kg) remained unaccounted for at the end of the experiment (probably due to nocturnal predation by herons) only collected mortalities were used in the LD_{50} calculation.

Dose–response and time-course studies

Treatment of experimental animals. Rainbow trout of 170–200 mm TL and from 50 to 75 g in weight were

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Table 1. Review of fish vomiting reports

Causative agent or treatment	Source	Taxon	Presence of vomiting	Materials vomited
Tartar emetic:				
1 mg/ml oral (p.o.)	Jernejcik (1969)	*Micropterus salmoides*	+	minnow and goldfish species
1 mg/ml p.o.	Daley *et al.* (1981)	*Cottus* spp.	+	invertebrates, fish
2 mg/ml p.o.	Foster (1977)	*Esox americanus*	+	invertebrates, fish
2 mg/ml p.o.	Foster (1977)	*M. salmoides*	+	invertebrates, fish
Arsenous acid:				
1 mg/ml p.o	Markus (1932)	*M. salmoides*	+	fish
1 mg/ml p.o.	Jernejcik (1969)	*Stizostedion vitreum*	+	minnows
Apomorphine:				
0.1 to 8.0 mg/ml i.p.	Jernejcik (1969)	*M. salmoides*	+	fish
0.1 to 0.2 mg/ml on gills	Jernejcik (1969)	*M. salmoides*	—	—
Immersion in 10% formalin.	Webster (1942)	*Morone americana*	+	misc. food items
	Schreiber and Minckley (1981)	*Catastomidae* spp.	—	—
		Cyprinidae spp.	—	—
	Treisman (1977)	*Gadidae* sp.	+	?
Motion sickness:				
Copper sulphate:				
6% p.o.	Tiersch (1986)	*Cottus bairdi*	+	invertebrates
6% p.o.	Tiersch (1986)	*Salmo gairdneri*	+	plastic balls
Immersion in lethal concentration of MS-222 anesthetic	J. W. Hilton*	*S. gairdneri*	+	food pellets
Gelatin capsule gavage:	McLaren (1946)	*S. gairdneri*	+	gelatin capsules
Evaluation of grain contaminant vomitoxin in pelleted food:	Woodward (1983)	*S. gairdneri*	—	—

*Personal communication 1985.
+, Presence of vomiting.

Table 2. 7-Day survival of rainbow trout following a single intraperitoneal injection of apomorphine–HCl

Apomorphine–HCl dose (mg/kg) i.p. or treatment	n	Number of fish surviving (hr after injection) 2	12	24	72	168
Vehicle control	14*	15	15	15	14	14
20	15	15	15	15	14	14
50	14*	15	15	15	14	14
100	14*	15	12	11	10	10
200	15	6	6	6	6	6
400	15	4	2	2	1	1

*A single fish disappeared from each of these groups during the night between 24 and 36 hr after injection.

transported to 300-l aerated holding tanks in a cold room kept at 16–17°C with a 16L:8D photoperiod schedule, and kept at least 1 week prior to use in experiments. Food was withheld for 24 hr before testing. Since prior work had shown that observations could be made effectively on groups of fish, treatments were performed on several fish simultaneously. The fish were marked, weighed, measured and transferred in groups of three or four to a terminally screened, 60-l observation section in a custom-made 275-l recirculating stream tank. A current of 5.8 ± 2.0 cm/sec mean velocity, which elicited a positive rheotactic response, was created by a bubbler airlift system (Lawson, 1982). Fish were allowed to acclimate for at least 6 hr and baseline observations were made through the clear plexiglas tank wall.

Ball insertion and injections. Each fish was recaptured and held loosely in a hand net while two 6 mm diameter, soft, plastic balls were inserted individually through the esophagus and pushed with a rod into the cardiac portion of the stomach. Ball placement could be judged by a combination of esophageal resistance and lateral palpation. The balls were bright yellow and individually numbered for fish identification. The fish were then injected and returned to the observation section of the tank. Five drug treatment groups (30, 60, 80, 100, and 120 mg/kg) were employed, as well as saline-injected and handling (non-injected) controls. Apomorphine–HCl solutions were prepared in purified distilled water, kept in foil-wrapped flasks, and used within 30 min. All injections were made with a 25 ga needle on the right side of the fish, immediately anterior and lateral to the anus. Dose volumes were calculated as 0.4% of body mass (e.g. 0.2 ml for a 50 g fish) and ranged between 0.2 and 0.3 ml. Physiological saline (0.67%) was used as an injection control.

Vomiting behavior. Vomiting behavior (VB) was defined in this study as an assemblage of behaviors which occurred simultaneously. The actual components were lowering of the gular pouch (a distensible area below the lower jaw), hyperexpansion of the mouth, pronounced opercular flaring and forceful lateral head movements. These motions were characterized by an almost tetanic increase in muscle tone and could occur as a single event of about a second in duration. The expulsion of gastrointestinal contents through the mouth as a result of VB was termed vomiting or emesis.

Collection of data. During the acclimation period, baseline movement, respiration and behavioral data were collected. Respirations, as evidenced by coordinated mouth and gill movements, were tallied for 20-sec intervals. Following injections, behavior, respiration rates and general appearance were monitored and recorded over a 2.5-hr period. Individul episodes of vomiting and VB were tallied for each fish as well as the onset time for these behaviors. At the end of 2.5 hr the fish were killed and dissected to verify the location of the plastic balls in the gut and the site of drug injection. Gastric pH was measured using pH paper which had been previously tested to 0.1 pH units with a pH meter. Sufficient fluid for accurate pH readings was not available in all stomachs and thus seven fish were excluded from pH analysis.

RESULTS

LD_{50}

Apomorphine, in doses above 200 mg/kg, is quite toxic to rainbow trout (Table 2). The LD_{50} value for the 7-day study was 158 mg/kg. Seventy-four percent of total mortality occurred in the first 2 hr. Eighty-nine percent was observed within 12 hr after injection of the drug, and no fish died after 72 hr. Fish that died within the first 2 hr displayed listing movements from side to side, followed by convulsions and violent propulsive motions which sometimes caused them to leap out of the water. Later, ataxia, loss of righting ability and respiratory depression were observed. Overall, 69% of the fish survived the treatments.

Dose–response and time-course studies

Vomiting. Apomorphine caused vomiting as well as vomiting behavior (VB is defined as all the behaviors associated with vomiting, but lacking ejection of the plastic balls). Vomiting behavior was never observed in fish of the saline or handling control groups but it was observed in 92% of the apomorphine-injected fish (Table 3). Approximately 30 VB occurrences were observed per fish during an hour period but the number of times each fish displayed VB was not dose related. Five fish vomited and one fish from the 100 mg/kg group ejected both plastic balls. A moribund fish in the 60 mg/kg group vomited a pellet 51 min after drug injection but held the ball passively in its mouth for the remainder of the observation period, apparently being too weak to fully eject the ball. Four of the five fish that vomited exhibited bouts of VB prior to vomiting. Mean VB onset times decreased in a dose-related fashion in all but the 120 mg/kg group where a single high value (due to

Table 3. Vomiting behavior (VB), vomiting and mortality following intraperitoneal injection of apomorphine–HCl in rainbow trout

Apomorphine–HCl intraperitoneal dose (mg/kg) or treatment	n	VB	Time (min) after injection until initial vomiting behavior $\bar{x} \pm$ SE	Minimum + maximum	No. vomiting	Time (min) after injection until vomiting occurred	No. dead in 2.5 hr
Handling control	10	0	—	—	0	—	0
Saline control	10	0	—	—	0	—	0
30	5	4	47 ± 19.7	23, 110	0	—	0
60	5	5	34 ± 8.9	28, 140	1	51	0
80	5	4	24 ± 5.4	15, 42	0	—	0
100	5	5	14 ± 1.8	11, 20	2	107, 138	1
120	5	5	35 ± 21.0	8, 105	2	16, 105*	1

*No prior vomiting behavior observed.

Table 4. Gastric pH following intraperitoneal apomorphine–HCl injections in rainbow trout. Handling controls received no injections

Apomorphine–HCl intraperitoneal dose (mg/kg) or treatment	n	Gastric pH ≥4.0	pH $\bar{x} \pm$ SE	pH minimum and maximum
Handling control	10	0	2.5 ± 0.2	1.5, 3.5
Saline control	5	0	2.5 ± 0.3	1.5, 3.5
30	3	2	5.2 ± 1.3	2.5, 6.5
60	2	2	5.5 ± 1.5	4.0, 7.0
80	3	3	5.7 ± 1.2	4.0, 8.0
100	5	5	6.6 ± 0.2	6.0, 7.0
120	5	5	7.3 ± 0.4	6.0, 8.5

a fish that vomited at 105 min with no previous observed VB) skewed the average.

Respiratory effects. Respiratory rate was increased in the fish that were injected with apomorphine. Respiration rates in all fish were high after handling, typically 105–120 respirations/min (res/min). By 9 min after the injection of saline (or handling) control animals decreased to 90–105 res/min. On the other hand, the respiration rates of the fish treated with apomorphine continued to increase and all were higher than control values after 9 min. Fifteen minutes after the fish were injected with apomorphine there were greater than or equal to 120 res/min while the control rates were about 90 res/min. Some apomorphine-treated animals actively involved in agonistic behavior had respiratory rates of over 150 res/min. During the remainder of the observation period a difference of at least 30 res/min was always present between the control animals and the apomorphine-injected fish.

Effects on motor control. Apomorphine caused varying degrees of impaired motor control and balance at all doses used in this study. After being injected, all fish rested on the tank bottom. Within 10 min, controls were up and swimming normally. The apomorphine-treated animals however, typically remained on the bottom for 35–40 min and were observed lowering their gular pouch and rocking from side to side as early as 8 or 9 min after drug injection. Occasionally fish were almost completely on their side prior to righting attempts. Most fish appeared to support themselves on the substrate with their pectoral fins or rested against the tank wall for support. Fish were also observed leaning against one another and this seemed to induce spawning-like shivering motions in some. Large increases in body tone were noted in some fish that were observed arching upward or laterally in a tonic spasm.

Effects on aggressive behavior. Apomorphine appeared to cause aggressive behavior in some fish, usually starting about 35–45 min after treatment. This was evidenced by attacks, nipping, chasing and lateral and frontal displays. Dominant and submissive roles were assumed; the more aggressive fish were often seen bumping and rubbing their noses on the tank wall, presumably attacking their own reflections. It was not uncommon for a smaller fish to become the most aggressive fish in the tank and assume a top position in a dominance hierarchy. Controls did not display this aggression and were only occasionally chased or attacked. Overall, this behavior was episodic and between bouts the fish returned to the tank bottom.

Effects on gastric pH. Apomorphine increased gastric pH. Gastric pH values of 4.0 or greater were found in 17 out of 18 apomorphine-injected fish for which pH was measurable (Table 4). All 15 control fish had gastric pH values of 3.5 or less.

DISCUSSION

Apomorphine induced a predictable assemblage of behaviors in rainbow trout, that were defined as vomiting and vomiting behavior using a framework adapted from that proposed for mammals by Borison and Wang (1953). The lowering of the gular pouch was an early and reliable indicator of subsequent vomiting behavior. A mechanical technique (the use of a balloon to distend the anterior intestine) has been found to clearly result in gular pouch expansion and VB in rainbow trout (Tiersch, 1986). This observation agrees with results of similar experiments in mammals (Borison and Wang, 1953) where distention of the intestine resulted in vomiting behavior and vomiting.

Vomiting behavior was distinct and could be clearly differentiated from other actions. A normal behavior was noted in fish in which air bubbles were expelled through the mouth after swimming to the surface, or when a fish was clearing debris from its throat or gills taken in during respiratory motions. This behavior was referred to as "coughing" and was not considered vomiting behavior. Convulsions were observed in apomorphine-injected trout, and despite being powerful motor activities, were much more erratic and less coordinated than VB, and could thus also be differentiated from vomiting behavior. Vomiting behavior onset times, although variable, showed a definite dose-related trend. No control fish exhibited VB and none vomited. The lowest dosage to induce vomiting was 60 mg/kg, although VB was observed at half this dose.

Plastic balls were used to establish the presence of emesis but did not seem to possess the same apomorphine dose–response profile as voluntarily ingested foot items. We have seen the emesis of food pellets, for instance at lower doses (unpublished data). The apparent low rate of emesis of plastic balls could possibly be related to their size, texture, shape or method of gastric introduction. Since the LD_{50} was calculated to be 158 mg/kg and a dosage of 120 mg/kg yielded vomiting in only two out of five fish (along with a mortality), it does not seem possible to produce higher vomiting rates for the plastic balls without also increasing mortality.

The controls might have been expected to vomit, given the responses noted in gelatin capsule gavage

(Table 1) by other workers (McLaren *et al.*, 1946), but the plastic balls may have been smaller or less irritating than the gelatin capsules. Two fish (one control, and one receiving apomorphine) did vomit a plastic ball in less than 30 sec after injection. Whether this was a voluntary response to remove a ball lodged in the esophagus (which in trout, contains skeletal muscle), or an immediate vomiting response is not known, but these fish were excluded from the study.

Apomorphine apparently affected the functioning of the central nervous system in rainbow trout, causing responses such as loss of equilibrium, ataxia, tonic spasms, convulsions and respiratory manifestations. Non-lethal administrations increased respiratory rates while respiratory depression and arrest were associated with high doses and death. The apomorphine-related agonistic behavior may be due to action at a central "aggression center". This was viewed, however, as a consequence of gular pouch lowering and opercular flaring, components of VB, and also lateral and frontal displays, which could have acted as releasers for aggression. Interestingly, the increased respiratory rates and atypical dominance hierarchies caused by apomorphine both disappeared at the same time; about 12–14 hr after injection (unpublished data). Increases in aggression, respiratory rates, and "coughing" (possibly vomiting) behavior were also found in bluegill that were exposed to copper sulphate (Henry and Atchison, 1986), a chemical that also caused vomiting in rainbow trout (Tiersch, 1986).

Morphine and related drugs can cause a decrease in gastric secretion of hydrochloric acid (Jaffe and Martin, 1980). The dose-related increase in gastric pH noted in the apomorphine injected groups indicates a similar response in rainbow trout. Further work might include a time course for this phenomenon and elucidation of its mechanism.

Apomorphine causes vomiting in mammals through direct stimulation of a chemoreceptor trigger zone located on the floor of the fourth ventricle in the area postrema of the medulla. This zone stimulates a brain vomiting center which coordinates emesis (Wang and Borison, 1952). Our results suggest that trout may possess a chemoreceptor trigger zone and brain vomiting center similar to those in mammals. Future work might compare trout vomiting mechanisms to those of other vertebrates using voluntarily consumed food items. The trout may prove useful for biomedical research as an emetic model not subject to the influences of mammalian higher brain centers. The exaggerated body motions and increased respiratory rates caused by apomorphine, may be similar to the chemically-induced behaviors that provide the basis for automated biomonitoring of water quality (Morgan *et al.*, 1981; Cairns and Garton, 1982). Systematic research in fish emesis may furnish "ichthyoemetics" for use as an adjunct administered prior to existing techniques such as pulsed gastric lavage (Light *et al.*, 1983; Foster, 1977) for collection of gastric contents from live fish.

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