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42. Dynamics of MS-222 in the Blood and Brain of Freshwater Fishes During Anesthesia

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DYNAMICS OF MS-222 IN THE BLOOD AND BRAIN OF FRESHWATER FISHES DURING ANESTHESIA

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ABSTRACT.--Eleven species of freshwater fishes were rapidly anesthetized in solutions of MS-222 containing from 100 to 1,000 milligrams of MS-222 per liter. MS-222 concentrations in blood and brain after 1 minute of exposure indicate that MS-222 rapidly diffuses across the gill and passes the blood-brain barrier. Evidence of metabolism of the drug was seen in the presence of acetylated MS-222 in the blood of all species studied. The concentration of free MS-222 in the brain increased with depth of anesthesia to loss of reflex and then either increased or declined slightly as the fish approached medullary collapse.

MS-222 (methanesulfonate of meta-aminobenzoic acid ethyl ester) is an effective fish anesthetic when administered by immersing fish in a solution or by spraying it on their gills (Schoettger, 1967). In either case, the route of entry is the gills. MS-222 is a lipid-soluble compound which is only 0.01-percent ionized at body pH (Maren, Embry, and Broder, 1968). This lipid solubility most likely accounts for its rapid diffusion across the gills.

Once the drug enters the bloodstream, it is distributed throughout the body. Although the site of action of MS-222 has not been established, it is thought to be in the brain. The blood-brain barrier in fish is known to exclude certain dyes, such as sulfonilic acid, from the cerebrospinal fluid (Rall, 1967). Preliminary investigations by Stenger and Maren (1968) indicate that MS-222 effectively crosses this barrier in the dogfish shark (Squalus acanthias). My studies were designed to extend this observation by measuring the rate of uptake of MS-222 in blood and brain of freshwater fish during the induction of anesthesia.

METHODS AND MATERIALS

Eleven species of fish were obtained from several sources (table 1). All specimens were maintained according to the methods of Hunn, Schoettger, and Whealdon (1968), except carp

Table 1.--Sources and sizes of fish used in the MS-222 uptake studies

Species	Length (inches)	Weight (grams)	Source
Shortnose gar *Lepisosteus platostomus*	21.5-27.5	--	Mississippi River Guttenberg, Iowa
Longnose gar *Lepisosteus osseus*	27.0-33.5	--	Mississippi River Guttenberg, Iowa
Bowfin *Amia calva*	23.0-31.0	--	Mississippi River Guttenberg, Iowa
Rainbow trout *Salmo gairdneri*	11.0-16.0	296-720	National Fish Hatchery Manchester, Iowa
Northern pike *Esox lucius*	10.0-18.0	--	Mead Wildlife Area Marshfield, Wis.
Carp *Cyprinus carpio*	8.5-10.8	140-350	Mississippi River Genoa, Wis.
Spotted sucker *Minytrema melanops*	11.8-14.3	--	Mississippi River Guttenberg, Iowa
Black bullhead *Ictalurus melas*	7.0-9.6	84-220	Mead Wildlife Area Marshfield, Wis.
Channel catfish *Ictalurus punctatus*	9.8-15.8	104-540	Mississippi River Lansing, Iowa
White bass *Roccus chrysops*	12.2-14.0	435-620	Mississippi River Guttenberg, Iowa
Bluegill *Lepomis macrochirus*	7.5-9.3	197-345	National Fish Hatchery Fairport, Iowa

and black bullheads, which were held at 17° C. The anesthetic solution of MS-222 in well water was made up fresh daily. The MS-222 was technical grade (99.4 percent) methanesulfonate of m-aminobenzoic acid ethyl ester obtained from Sandoz Pharmaceuticals. Desired concentrations of the drug were achieved by adding the crystaline material to measured volumes of well water in 5-gallon stainless-steel pails or in 45- or 100-liter polyethlene tanks. Individual fish were immersed in the anesthetic solution for periods of 1, 3, 5, 8, or 11 minutes. All fish were anesthetized to loss of reflex, and most were nearing medullary collapse in 8 to 11 minutes of exposure.

Blood samples were taken by caudal puncture (Steucke and Schoettger, 1967). The spinal cord of the fish was then severed and the brain removed. Concentrations of MS-222 and background primary aromatic amines in whole blood and brain were determined by the Bratton-Marshall method as modified by Walker and Schoettger (1967). The average concentration of background amines was subtracted from total aromatic amines to determine the concentration of MS-222.

RESULTS

MS-222 moves rapidly across the gills and enters the bloodstream of fishes (table 2). Within 1 minute of exposure, the drug concentration greatly exceeds the background level of primary aromatic amines. The ratio of the highest average concentration of MS-222 in whole blood to that of the anesthetic solution ranged from 0.14 in shortnose gar to 0.83 in rainbow trout.

Background primary aromatic amines in whole blood ranged from 0.6 to 5.4 milligrams per liter (mg/l) as free amines, and 0.0 to 4.0 mg/l as acetylated amines.

In seven of the eleven species, the brain concentration of MS-222 exceeded that of the whole blood after the first minute of exposure (table 2). The brains of all species contained amounts of MS-222 in excess of those in the blood after 3 minutes.

Table 2.--Concentration of MS-222 in whole blood and brain of 11 species of fish during the induction of anesthesia

[Condition of anesthesia for each species listed in table 3]

Species and exposure time	Concentration in ppm									Brain-blood ratio
	Whole blood						Brain			
	Free MS-222			Acetylated MS-222			Free MS-222			
	n	Mean	Range	n	Mean	Range	n	Mean	Range	
Shortnose gar:										
0 min.[1]	2	0.6	0.6	2	1.8	1.0-2.6	2	3.6	3.2-4.0	6.0
1 min.	2	143.2	93.8-192.6	2	10.3	0.0-20.6	2	135.6	128.8-142.4	0.95
3 min.	2	135.0	135.0	2	8.2	7.8-8.6	2	342.8	301.6-384.0	2.54
5 min.	2	108.2	105.4-111.0	2	8.8	8.6-9.0	2	269.6	238.4-300.8	2.49
8 min.	2	122.8	95.0-150.6	2	0.9	0.0-1.8	2	230.4	190.8-270.0	1.88
Longnose gar:										
0 min.[1]	2	2.1	1.6-2.6	2	1.2	0.6-1.8	2	2.9	2.4-3.4	1.38
1 min.	1	74.7	-	1	2.8	-	2	88.3	58.8-117.8	1.18
3 min.	2	135.3	126.4-144.2	2	8.5	0.0-17.0	2	265.1	221.6-308.6	1.96
5 min.	2	114.3	110.0-118.6	2	1.7	0.0-3.4	2	277.5	262.4-292.6	2.43
8 min.	2	127.1	124.8-129.4	2	8.0	5.8-10.2	2	228.7	225.6-231.8	1.80
Bowfin:										
0 min.[1]	2	3.9	3.2-4.6	2	0.2	0.0-0.4	2	8.6	7.2-10.0	2.21
1 min.	2	202.2	163.6-240.8	2	105.1	75.0-135.2	2	58.3	45.6-71.0	0.29
3 min.	2	178.5	166.6-190.4	2	64.0	6.6-121.4	2	217.3	157.8-276.8	1.22
5 min.	2	186.0	184.6-187.4	2	37.0	34.2-39.8	2	188.8	184.8-192.8	1.02
8 min.	2	117.3	100.0-134.6	2	0.0	-	2	222.8	192.8-252.8	1.90

See footnotes at end of table.

Table 2.--Concentration of MS-222 in whole blood and brain of 11 species of fish during the induction of anesthesia--Continued

Species and exposure time	Concentration in ppm									
	Whole blood						Brain			Brain-blood ratio
	Free MS-222			Acetylated MS-222			Free MS-222			
	n	Mean	Range	n	Mean	Range	n	Mean	Range	
Rainbow trout:										
0 min[1]	8	1.7	1.3-2.8	8	0.7	0.4-1.1	12	3.2	2.3-5.6	1.83
1 min	4	69.2	43.3-104.2	4	3.8	0.6-7.9	8	116.0	107.7-125.7	1.68
2 min	5	51.9	42.7-66.8	5	3.1	0.6-6.9	8	145.1	136.8-150.7	2.80
4 min	5	68.6	49.9-82.2	5	1.7	0.0-5.3	8	165.4	159.1-169.2	2.41
6 min	4	68.5	60.9-72.6	4	2.0	0.4-3.1	5	156.8	146.1-172.0	2.29
10 min	5	83.1	66.3-94.2	5	2.9	0.0-4.9	5	154.1	144.9-159.6	1.85
Northern pike:										
0 min[1]	6	1.9	1.0-2.4	5	0.7	0.4-4.0	[2]3	1.8	1.6-2.2	0.95
1 min	6	35.9	23.0-57.6	6	1.5	0.0-3.9	[2]3	66.9	60.6-78.4	1.86
3 min	6	81.9	60.4-111.6	5	3.4	1.3-5.3	[2]3	152.9	140.8-158.6	1.87
5 min	6	86.0	73.0-100.4	5	13.1	4.0-16.8	[2]3	204.6	183.6-223.8	2.38
8 min	6	95.7	83.0-105.6	6	7.7	2.4-11.2	[2]3	248.1	230.4-257.8	2.59
Carp:										
0 min[1]	6	1.4	1.2-2.0	6	0.7	0.0-2.8	[2]3	1.1	1.0-1.4	0.79
1 min	6	63.9	34.4-82.0	6	3.8	0.0-17.2	[2]3	54.6	44.2-66.6	0.85
3 min	6	96.0	78.4-114.0	6	2.5	1.4-2.8	[2]3	164.1	155.8-171.4	1.71
5 min	6	90.2	82.8-97.8	6	17.2	3.4-29.8	[2]3	165.8	151.4-192.6	1.84
8 min	6	100.8	88.8-115.6	5	1.7	0.0-2.6	[2]3	190.0	187.0-192.6	1.88
11 min	6	89.5	72.4-105.0	6	3.5	0.0-6.3	[2]3	156.5	134.6-176.2	1.75
Spotted sucker:										
0 min.[1]	6	1.8	1.6-2.2	6	0.0	-	[2]3	4.9	4.0-6.6	2.7
1 min	2	113.4	86.2-140.6	2	0.0	-	[2]P	35.5	-	0.3
3 min	2	100.3	66.0-134.6	2	10.7	7.0-14.4	[2]P	143.7	-	1.4
5 min	2	108.9	58.8-159.0	2	6.6	6.4-6.8	P	190.0	-	1.7
8 min	2	121.0	103.8-138.2	2	0.7	0.0-1.4	P	200.3	-	1.6
11 min	2	102.7	83.2-122.2	2	1.5	1.0-2.0	P	184.1	-	1.8
Black bullhead:										
0 min.[1]	8	2.3	1.6-3.0	8	0.2	0.0-0.6	[2]4	4.8	4.0-5.5	2.09
1 min	6	68.8	42.0-148.0	6	0.4	0.0-2.8	[2]3	111.3	102.8-119.6	1.62
3 min	5	121.5	102.6-174.2	4	23.1	0.0-62.2	[2]3	162.8	140.3-185.7	1.34
5 min	6	145.5	109.0-197.0	5	19.3	12.0-27.4	[2]3	227.1	194.4-259.7	1.56
8 min	6	146.8	130.4-165.8	6	10.9	1.2-15.4	[2]3	266.9	252.0-284.5	1.82
11 min	6	127.9	110.0-156.2	6	11.1	8.0-15.4	[2]3	240.5	220.0-250.5	1.88
Channel catfish:										
0 min.[1]	16	1.2	0.6-1.8	16	1.2	0.6-1.6	8	3.9	2.6-4.6	3.25
1 min	4	114.4	104.4-120.6	4	3.2	0.0-8.2	4	188.6	178.1-198.1	1.65
3 min	4	137.3	123.8-164.0	4	11.7	6.2-14.2	4	260.3	239.7-290.5	1.89
5 min	4	116.4	99.8-120.4	4	11.4	0.8-19.6	4	235.6	227.7-243.7	2.02
8 min	4	106.7	94.0-111.0	4	7.6	2.2-9.8	3	182.3	150.7-240.2	1.71
11 min	4	115.2	104.2-140.8	4	9.2	5.6-13.2	4	217.9	184.5-258.6	1.89
White bass:										
0 min.[1]	3	4.1	3.4-5.4	3	0.4	0.0-1.2	3	2.7	2.0-3.8	0.65
1 min	2	42.8	39.7-45.9	2	3.8	3.6-4.0	2	47.7	45.1-50.3	1.11
3 min	5	86.7	44.3-102.5	5	10.1	2.8-14.4	5	109.7	77.1-132.9	1.27
5 min	2	90.3	84.7-95.9	2	21.2	14.8-27.6	2	115.1	104.9-125.3	1.27
8 min	2	88.9	86.3-91.5	2	13.8	12.8-14.8	2	130.9	125.3-136.5	1.47
Bluegill:										
0 min.[1]	6	1.9	1.4-2.6	6	0.9	0.0-2.2	[2]3	3.1	2.8-3.2	1.61
1 min	6	62.7	27.6-93.4	6	2.9	0.0-8.6	[2]3	89.0	67.6-109.6	1.42
3 min	6	104.3	87.2-123.0	5	5.1	0.0-9.2	[2]3	168.2	152.4-189.2	1.61
5 min	12	121.8	87.7-134.1	11	12.8	0.0-63.9	[2]6	174.2	129.3-206.5	1.43
8 min	6	98.5	91.0-104.6	6	17.5	12.0-20.6	[2]3	196.5	180.0-208.0	1.99
11 min	6	95.5	74.2-117.0	6	8.7	0.0-14.6	[2]3	174.2	163.6-179.6	1.82

[1]Background level of primary aromatic amines.
[2]P = pooled sample, 2 brains per sample.

Table 3.--Concentrations of MS-222 in blood and brain of 11 species of fish at loss of reflex stage of anesthesia

Species	Temperature °C.	Anesthetic concentration (mg/l)	Time in anesthetic at loss of reflex (minutes)	Average concentration of free MS-222[1]	
				In whole blood (mg/l)	In brain (mg/kg)
Shortnose gar	12	1,000	2-3	135.0	342.8
Longnose gar	12	800	2-3	135.0	265.1
Bowfin	12	1,000	2-3	178.0	217.3
Rainbow trout	12	100	3-4	68.6	165.4
Northern pike	12	150	2-3	81.9	152.9
Carp	17	200	3-4	96.0	164.1
Spotted sucker	12	200	2-3	100.3	143.7
Black bullhead	17	200	5-6	145.5	227.1
Channel catfish	12	200	2-3	137.3	260.3
White bass	12	150	2-3	83.6	107.1
Bluegill	12	200	2-3	104.3	168.2

[1] Average concentrations of free MS-222 compiled from table 2.

A minimum concentration of 100 milligrams per kilogram (mg/kg) of free MS-222 appears to be necessary for anesthesia to loss of reflex judging from the average concentrations measured in the brain of 11 species (table 3).

DISCUSSION

Diffusion of MS-222, a highly lipid-soluble nonpolar drug, across the gills of fish is quite rapid. Movement of the drug may be in either direction depending on the concentration gradient. This study has shown concentrations of MS-222 in both blood and brain greatly in excess of background amines after 1 minute of exposure to the anesthetic solution. As shown by Maren, Embry, and Broder (1968) in their study on the dogfish shark, the gill is quite efficient in clearing the blood of MS-222 during recovery from anesthesia. Hunn, Schoettger, and Willford (1968) have indirectly measured the same phenomenon in rainbow trout. Preliminary investigations by Maren, Broder, and Stenger (1968) showed that the nonpolar ethyl m-aminobenzoate and its N-acetyl derivative are both excreted across the gill while the polar m-aminobenzoic acid and its N-acetyl derivative are excreted via the kidney. Most of the MS-222 and its congeners are excreted via the gills during recovery; 95 percent in the dogfish shark (Maren, Embry, and Broder, 1968) and 79 to 85 percent in the rainbow trout (Hunn, Schoettger, and Willford, 1968).

Concentrations of MS-222 in whole blood (table 2) drawn via caudal puncture did not reach the levels in the anesthetic solutions during exposures as long as 11 minutes (fish approaching medullary collapse). This is probably due to the fact that blood drawn by this method is usually venous blood which would contain a lesser concentration of the drug than arterial blood until the drug is in equilibrium between the fish and the anesthetic solution.

The appearance of acetylated MS-222 in most blood samples indicates that all 11 species are able to metabolize it. Highest concentrations of acetylated drug were usually detected after 3 to 5 minutes of exposure. The bowfin had the greatest blood concentration of acetylated MS-222 of any of the 11 species studied, 34 percent after a 1-minute exposure. Concentrations in the 10 species were usually less than 20 percent. Maren, Broder, and Stenger (1968) found the same level of acetylated drug in the plasma of the dogfish shark during recovery from anesthesia.

Stenger and Maren (1968) reported that during MS-222 anesthesia of the dogfish shark, the drug rapidly reaches the cerebrospinal fluid and the brain. My observations confirm this finding. In all 11 species, the concentration of free MS-222 in the brain was significantly above background after 1 minute of exposure. The concentration of drug in the brain increased with depth of anesthesia to loss of reflex. With deeper anesthesia, the concentration of free MS-222 either increased slightly or declined in comparison with the concentration at loss of reflex. A concentration of at least 100 mg/kg is necessary for anesthesia to loss of reflex in susceptible species like rainbow trout, whereas the more resistant species like black bullhead require approximately 200 mg/kg of the free drug for a similar level of anesthesia.

In a previous paper (Hunn, 1968) I noted that rapid recovery in fresh water is associated with the declining concentration of free MS-222 in the brain of channel catfish. The brain concentration of free drug was 91.6 mg/kg when the catfish righted themselves whereas it was 260.3 mg/kg when they exhibited loss of reflex. Indeed, in all studies published to date anesthesia and recovery in fresh water have been strictly associated with the concentration of free drug in the blood and brain (Schoettger et al., 1967; Walker and Schoettger, 1967b).

SUMMARY

Eleven species of freshwater fish were rapidly anesthetized in solutions of MS-222 containing from 100 to 1,000 mg/l of drug. MS-222 (free and acetylated) concentrations in whole blood and brain after 1 minute of exposure indicate that MS-222 rapidly diffuses across the gill and passes the blood-brain barrier. Blood samples drawn by caudal puncture contained lower concentrations of MS-222 than those of the anesthetic solutions.

The presence of acetylated MS-222 in the blood of all species studied is evidence that fish metabolize the drug. Concentratins of acetylated drug were usually less than 20 percent of the total MS-222 except those in bowfin which had 34-percent acetylation after a 1-minute exposure.

MS-222 rapidly enters the brain from the blood. The concentration in the brain increases with depth of anesthesia to loss of reflex. As fish enter more deeply into anesthesia, the drug concentration either increases slightly or declines in ratio to the levels at loss of reflex. Anesthesia and recovery in fresh water appears to be associated with the concentration of free MS-222 in the blood and brain.

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