Pharmacology Biochemistry and Behavior, Vol. 44, pp. 321-327, 1993

0091-3057/93 $6.00 + .00

MAO-A and -B Inhibitors Selectively Alter *Xenopus* Mucus-Induced Behaviors of Snakes

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Received 20 February 1992

BARTHALMUS, G. T., L. K. HARDIN AND D. THOMPSON. *MAO-A and -B inhibitors selectively alter* Xenopus *mucus-induced behaviors of snakes.* PHARMACOL BIOCHEM BEHAV **44**(2) 321-327, 1993.—Skin mucus of the frog *Xenopus laevis*, contacted orally by snakes, induces dyskinetic oral movements and climbing behavior that promote escape. The mucus contains peptides and indoleamines known to produce drug-induced movement disorders in other species. We hypothesized that inhibition of monoamine oxidase-A (MAO-A) by *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine [clorgyline (CLG)] and MAO-B by *R*(-)-*N*,*a*-Dimethyl-*N*-2-propynyl-benzeneethanamine [L-deprenyl (LDL)] would selectively modify mucus-induced behaviors by elevating norepinephrine and serotonin (with CLG), phenylethylamine (with LDL), or dopamine (with both drugs). In Experiment 1 (EXP1), adult snakes received mucus and/or 20 μg/g (IP) of both drugs. In EXP2, juveniles received mucus and/or 5, 10, and 20 μg CLG or LDL. CLG given alone had no effect on tongue flicking, activity, and climbing (EXP1,2). LDL alone decreased tongue flicking in EXP2 and increased climbing (EXP1,2). Given with mucus, both drugs further lowered the tongue flicking rates attenuated by mucus (EXP1,2); only LDL potentiated mucus-induced climbing. Yawning was the only mucus-induced dyskinesia attenuated (20 μg CLG, adults; 20 μg LDL, juveniles). We suggest that dopamine and/or phenylethylamine, the substrates for MAO-B, may promote mucus-induced climbing and tongue flicking but may have some protective role against mucus-induced yawning in water snakes.

Xenopus skin mucus MAO-A MAO-B Monoamine oxidase Deprenyl Clorgyline Snakes Movement disorders Parkinsonism

OUR laboratory has been developing a unique animal model for studying neuroleptic- and neurotoxin-induced movement disorders such as dyskinesias, dystonias, and Parkinsonism. Just as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced abnormalities now serve as a model for studying motor symptoms of Parkinson's disease [(21,22) for reviews], orofacial dyskinesias induced in snakes by skin mucus of the clawed frog *Xenopus laevis* (1-3,38) also may serve as a model system for studying mechanisms of human movement disorders.

The secretory granules in *Xenopus'* granular skin glands contain caerulein (CRL) [a potent analog of cholecystokinin octapeptide (CCK-8)], xenopsin (an analog of neurotensin), thyrotropin-releasing hormone (TRH), magainin-1 and -2 (potent antimicrobial peptides), serotonin [5-hydroxytryptamine (5-HT)], bufotenidine (BUF), and a variety of active peptide fragments (3,4). Neuroleptic properties have been documented in humans and other mammals for CCK-8 and CRL (6,37), TRH (5), and neurotensin (12).

To date, our laboratory has made no attempt to characterize the behavioral role of pure, isolated components in *Xenopus* skin mucus as they relate to the antipredatory behaviors seen in snakes. Considering the diversity of agents in the mucus and others not yet identified, we elected to first test for effects on neural receptors known to be involved in movement disorders and hypertension. We reported that the dopamine receptor blockers haloperidol and SCH23390 modify *Xenopus* mucus-induced orofacial dyskinesias in the water snake, *Nerodia sipedon*, in ways that are compatible with their effects on mammals (1,2). *Xenopus* mucus also induces climbing behavior in snakes (1,2,38); however, neither haloperidol nor SCH23390 affected climbing when given alone or with skin mucus. It was proposed that climbing may be the snake's way to behaviorally counteract the hypertensive properties of the mucus (1).

In this article, we describe the effects of the monoamine oxidase-A (MAO-A) and -B (MAO-B) inhibitors *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine (clorgyline) and *R*(-)-*N*,*a*-Dimethyl-*N*-2-propynyl-benzeneethanamine (L-deprenyl), respectively, on *Xenopus* mucus-induced orofacial dyskinesias and climbing in the water snake *Nerodia sipedon*. L-Deprenyl (Selegiline) is an MAO-B inhibitor that has been used as an adjuvant to L-dopa therapy (17,21) for treating early stages of Parkinson's disease. Further, it can prevent the

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Parkinsonism induced in rodents and primates by the neurotoxicant MPTP (16,17). As MAO-B is the primary form of MAO in the human brain (36), L-deprenyl presumably increases availability of nigrostriatal dopamine by preventing the oxidation of dopamine or by possibly increasing dopamine synthesis and synaptic persistence of endogenous dopamine (15). In mammals including humans, MAO-B inhibition does not induce the hypertensive crisis ("cheese effect") that can occur when MAO-A inhibitors such as clorgyline are used to treat human depression (7). While dopamine, tyramine, and tryptamine are equally good substrates for both enzymes, MAO-A is more selective for serotonin and norepinephrine; MAO-B is more selective for β-phenylethylamine and benzylamine (9,13,31,34). Further, as the peptides and indoleamines in *Xenopus* skin mucus have dopamine receptor blocking properties, and because MAO-A and -B use dopamine as a substrate but otherwise have preferred substrates, we predicted that the respective MAO-A and -B inhibitors clorgyline and L-deprenyl would selectively modify *Xenopus* mucus-induced behaviors in snakes.

METHOD

Two experiments (EXP1, EXP2) were conducted and the procedure for each is detailed below. Briefly, EXP1 involved 12 adult water snakes (*N. sipedon*), each administered separate 20-μg/g body weight doses of clorgyline and deprenyl (and the distilled water vehicle) in combination with orally applied porcine mucin or *Xenopus* skin mucus. Porcine mucin served as a control for the physical presence of mucus placed into a snake's mouth. EXP2 involved 16 juvenile sibling snakes each administered 5, 10, and 20 μg/g (and distilled water vehicle) of either drug in combination with porcine mucin or *Xenopus* mucus. Snakes, naive to drugs and *Xenopus* mucus, were reared in plastic cages fitted with newspaper bedding and a water bowl, fed frozen bluegill sunfish (adults of EXP1) or small live minnows (juveniles of EXP2) to repletion once each week, and maintained on a 14 L : 10 D photoperiod at ambient laboratory temperatures. Male *X. laevis* frogs (Xenopus I, Ann Arbor, MI), having a 4- to 5-cm body length, were reared in a flow-through tank at 18°C and a 12 L : 12 D photoperiod and fed pelleted *Xenopus* chow (Carolina Biol. Supply, Burlington, NC).

Drugs and Frog Mucus Preparation

L-Deprenyl HCl and clorgyline HCl were obtained from Research Biochemicals Inc. (Natick, MA). Both drugs were dissolved in distilled water (vehicle) and injected ventrolaterally into the posterior coelom. In EXP1, 20 μg drug/g body weight was delivered as 100 μg drug in 0.01 cc vehicle. In EXP2, 5, 10, and 20 μg drug/g body weight was delivered as 5, 10, or 20 μg drug per 0.01 cc vehicle. Granular gland mucus was collected by injecting 0.012 mg epinephrine, dissolved in 0.2 cc distilled water, into the dorsal lymph sac of *X. laevis*. To prevent rapid drying and thickening of the mucus, tapwater was first applied to a frog's back and to the spatula used for removing the mucus. Mucus was applied immediately to the dorsal surface of a snake's mouth; one frog served as the mucus donor for testing two snakes (one for each observer). Powdered porcine stomach mucin (Sigma Chemical Co., St. Louis, MO), mixed with distilled water to the consistency of fresh *Xenopus* mucus, served as the sham control for the physical presence of orally applied frog mucus.

Experiment 1 Procedure

Subjects (n = 12) were adult *N. sipedon* captured in Raleigh, North Carolina, and reared in the laboratory for 6 months prior to study from February through April 1990. Body weights at the beginning of the study (83–250 g) remained stable during the 8-week investigation. Trials occurred for 30 min in 40-1 aquaria containing 3 cm tapwater at 26°C. Each of two observers was assigned six different snakes. Table 1 shows the treatment schedule for drug and frog mucus over the 8-week study. On Tuesdays, six snakes were tested, three in one drug order and three in the reverse order. On Wednesdays, six additional snakes were tested but in the reverse order used on Tuesdays. Thus, both observers tested three snakes on each of the two orders of drug administration.

The weekday schedule was as follows: *On Monday*, vehicle or drug was given to the six snakes to be tested on Tuesday. *On Tuesday*, the first six snakes received their second vehicle or drug injection 30 min prior to testing and, immediately prior to testing, these snakes were administered oral doses of either frog mucus or porcine mucin; the second six snakes received their first injections. *On Wednesday*, the second group of six snakes was given the second injection, held for 30 min, given frog mucus or porcine mucin, and then tested immediately for 30 min. *On Thursday*, all snakes were fed. *On Friday*, all snake cages were changed. Thus, each of 12 snakes was given both drugs (20-μg dose) and the water vehicle alone or in combination with frog mucus or porcine mucin. One matched vehicle and one frog mucus control treatment were associated with each drug.

Experiment 2 Procedure

Sixteen sibling water snakes, born August 1990, were studied between February and April 1991. Body weights at the beginning of the study (18–32 g) remained stable during the 8-week investigation. Each of two observers was assigned eight snakes: two snakes given deprenyl at increasing doses of 5, 10, and 20 μg/g body weight and two given decreasing doses; two given increasing doses of clorgyline and two given decreasing doses (see Table 2). Thus, each observer tested both drugs at all doses and both dosing orders. Forward and reverse drug orders were used to determine if prolonged inhibition by deprenyl can occur in snakes as it does in humans and laboratory mammals (11,18,27,32). Snakes were administered either deprenyl or clorgyline along with porcine mucin (sham mucus) or *Xenopus* skin mucus. Drug was given 24 h as well as 30 min prior to the weekly 30-min behavioral testing session. EXP2 differed from EXP1 in that juveniles were studied at three doses of only one drug, while in EXP1 both drugs were tested in each adult snake but only at the 20-μg dose. Other than the above, experimental protocols were identical between EXP1 and EXP2.

Behaviors Recorded

Mucus-induced oral behaviors included: *gaping*—slight opening then closing of the mouth; *yawning*—wide opening then closing of the mouth, often with the head dorsoflexed; *fixed gaping*—gaping longer than 4 s; *fixed yawning*—yawning longer than 4 s; *writhing tongue*—prolonged writhing movements of the tongue; *chewing*—alternate raising and lowering of the right and left jaw maxilla. Other behaviors recorded included: the number of *tongue flicks*—normal, rapid protrusions of the tongue; *climbing*—minutes reared vertically on the aquarium wall; and *activity*—minutes actively moving in the aquarium.

Statistical Analysis

Experiment 1. A repeated-measures analysis of variance (ANOVA) was used to test for observer (A and B), drug (de-

TABLE 1

EXPERIMENT 1: DRUG-MUCUS TREATMENT ORDER FOR 12 SNAKES (101-112) TESTED IN AN 8-WEEK EXPERIMENT BY OBSERVERS A AND B

	Observer A or B, Snake Number, and Treatment			
	Tested Tuesdays		Tested Wednesdays	
Week	A (101-103)	B (104-106)	A (107-109)	B (110-112)
1	Veh-D, PMucin	Veh-C, PMucin	Veh-D, PMucin	Veh-C, PMucin
2	Veh-D, mucus	Veh-C, mucus	Veh-D, mucus	Veh-C, mucus
3	20D, PMucin	20C, PMucin	20D, PMucin	20C, PMucin
4	20D, mucus	20C, mucus	20D, mucus	20C, mucus
5	Veh-C, PMucin	Veh-D, PMucin	Veh-C, PMucin	Veh-D, PMucin
6	Veh-C, mucus	Veh-D, mucus	Veh-C, mucus	Veh-D, mucus
7	20C, PMucin	20D, PMucin	20C, PMucin	20D, PMucin
8	20C, mucus	20D, mucus	20C, mucus	20D, mucus

Veh-D and Veh-C refer to the distilled water vehicle injection for deprenyl (D) and clorgyline (C), respectively. PMucin and mucus refer to treatments in which porcine mucin and *Xenopus* skin mucus, respectively, were applied orally to snakes along with 20-μg/g body weight IP injections of D or C. Drug was administered both 24 h and 30 min prior to the 30-min test session.

prenyl or clorgyline), drug order (deprenyl or clorgyline given first), mucus (porcine mucin or frog mucus), and mucus-drug effects. As neither drug nor drug vehicles induced oral behaviors, only treatments in which frog mucus was used are presented. Here, "drug" effects were calculated using the snake $\times$ drug mean square as the error. As activity, tongue flicking, and climbing can occur as normal spontaneous behaviors, the effects of porcine mucin, frog mucus, drug, and mucus-drug combination were calculated using the snake $\times$ treatment mean square as the error. Pairwise comparisons of means were conducted only if the appropriate ANOVA F-test was significant. Here, the protected least significant difference (LSD; $\alpha = 0.05$) procedure and snake $\times$ treatment as the error was used.

Experiment 2. This was a randomized factorial experiment with three factors at two levels each (two drugs $\times$ two orders $\times$ two observers). Eight measures were taken on each snake. The repeated-measures factors were mucus (porcine mucin or frog mucus), dose of drug (0, 5, 10, 20), and drug order (increasing or decreasing dose). For each response variable, a multivariate repeated-measures ANOVA was used to test for drug, order, dose, and mucus effects. Main effects for the "between-snake" factors—drug, order, and drug $\times$ order—were tested using F-tests. "Within-snake" factors (dose and type of mucus) were tested using Wilks' Lambda. To compare each dose with the zero dose, the least squares mean for each dose was computed along with the standard error for the difference between the mean and the mean at zero dose. Bonferroni's multiple-comparison procedure was used to compare each set of three doses to its control.

TABLE 2

EXPERIMENT 2: DRUG-MUCUS TREATMENT ORDER FOR 16 SNAKES (1-16) TESTED FOR 8 WEEKS IN 30-min SESSIONS BY OBSERVERS A AND B

	Snake Number and Drug/Mucus Treatment Order			
	Tested Wednesdays		Tested Thursdays	
Week	1-4*	5-8	9-12	13-16
1	Veh-D, PMucin	20D, mucus	Veh-C, PMucin	20C, mucus
2	Veh-D, mucus	20D, PMucin	Veh-C, mucus	20C, PMucin
3	5D, PMucin	10D, mucus	5C, PMucin	10C, mucus
4	5D, mucus	10D, PMucin	5C, mucus	10C, PMucin
5	10D, PMucin	5D, mucus	10C, PMucin	5C, mucus
6	10D, mucus	5D, PMucin	10C, mucus	5C, PMucin
7	20D, PMucin	Veh-D, mucus	20C, PMucin	Veh-C, mucus
8	20D, mucus	Veh-D, PMucin	20C, mucus	Veh-C, PMucin

Veh-D and Veh-C refer to the distilled water vehicle injection for deprenyl (D) and clorgyline (C), respectively. PMucin and mucus refer to procine mucin and *Xenopus* skin mucus, respectively, which was applied orally to snakes along with vehicle, 5-, 10-, or 20-μg/g body weight IP injections of D or C. Drug was administered both 24 h and 30 min prior to the 30-min test session.

*Observer A tested odd-numbered and observer B tested even-numbered snakes.

In EXP1, the yawning, tongue flicking, activity, and climbing response variables were transformed to square roots to stabilize the variance. In EXP2, only data from yawning were transformed to square roots.

RESULTS

Experiment 1

No involuntary oral behaviors (gaping, fixed gaping, yawning, fixed yawning, chewing, writhing tongue) were induced by deprenyl, clorgyline, or drug vehicle given in combination with porcine mucin, so those data were not included in the ANOVA or Table 3. However, all treatments involving frog mucus, with or without the 20-μg/g body weight dose of deprenyl or clorgyline, induced dyskinetic oral behaviors. The LSD pairwise analysis was conducted only for yawning because of the significant main effects noted from the ANOVA, $F(47, 3) = 5.25$, $p < 0.01$. Here, 20 μg clorgyline significantly attenuated mucus-induced yawning (means per 30-min sessions were: mucus alone, 41.3; mucus and clorgyline, 22.2). Effects of 20 μg deprenyl on yawning were suggestive but not significant (mucus alone, 28.8; mucus and deprenyl, 12.7). Analyses for observer effects and drug order effects were not significant.

As the ANOVAs for tongue flicking, activity, and climbing were significant (Table 3), the LSD procedure was performed for each of these behaviors. Frog mucus significantly attenuated tongue flicking when compared to the matched vehicle treatment for both drug series, but only deprenyl significantly attenuated the already low rate of tongue flicking induced by frog mucus. Neither drug altered tongue flicking from its vehicle response rate.

Neither deprenyl nor clorgyline given alone modulated activity, and neither drug modulated the activity associated with *Xenopus* mucus administration.

Deprenyl and clorgyline differed in their effects on climbing. Whereas clorgyline neither induced climbing nor modulated the significant climbing induced by frog mucus, deprenyl alone induced significant climbing when compared to vehicle treatment. The climbing induced by deprenyl was equivalent to that of mucus-induced climbing. Further, deprenyl significantly potentiated mucus-induced climbing. Observer differences were not statistically significant across response variables.

Experiment 2

One snake tested with clorgyline died in the third week of the study, so analyses were based upon 15 snakes (8 given deprenyl; 7 given clorgyline). No orofacial behaviors were induced by clorgyline, deprenyl, or drug vehicle when given in combination with porcine mucin, so those data are not included in Table 4. Although all treatments that included frog mucus induced oral behaviors, only yawning was attenuated by drug. Here, only 20 μg deprenyl (increasing drug order) given to juveniles significantly attenuated *Xenopus* mucus-induced yawning (Bonferroni's procedure; mean for mucus alone, 54.0; mucus and 20 μg deprenyl, 17.8, $p < 0.02$). In the decreasing drug order, the number of yawns occurring from 20 μg deprenyl given with skin mucus (mean 26.6) was not significantly different from trials in which mucus was given with the vehicle (mean 32.8, $p = 0.54$). Thus, a strong drug carryover effect is suggested by the number of yawns occurring in the two orders of drug administration.

All doses of deprenyl given with porcine mucin significantly attenuated normal tongue flicking. These decreases in tongue flicking were well below the significant attenuation of tongue flicking that resulted when *Xenopus* mucus was given alone (Table 4). Also, the 10- and 20-μg doses of deprenyl given with *Xenopus* mucus significantly decreased tongue flicking to numbers well below those obtained when *Xenopus* mucus was given with vehicle. The decreasing drug order revealed that the attenuating effects on tongue flicking by initial high doses of deprenyl had a long-lasting effect that continued to the vehicle dose. Thus, an insignificant difference in the rate of tongue flicking was found when vehicle and porcine mucin was compared to vehicle and *Xenopus* mucus. Clorgyline given with porcine mucin had no effect on tongue flicking. Only the 20-μg dose of clorgyline given with *Xenopus* mucus significantly attenuated tongue flicking.

All doses of deprenyl significantly induced climbing when given with porcine mucin, and 10 μg deprenyl potentiated the significant climbing induced when *Xenopus* mucus was paired with vehicle. Again, snakes given the decreasing dose order revealed sustained drug effects that persisted when the vehicle dose was administered. This latter pattern was not noted for treatments involving clorgyline. Clorgyline given with porcine mucin failed to induce climbing; however, 5 μg clorgyline potentiated the climbing induced by *Xenopus* mucus.

Clorgyline had no significant effects on activity. Only when 20 μg deprenyl was given with porcine mucin (decreasing drug order) or with *Xenopus* mucus (increasing drug order) was

TABLE 3

RESULTS OF EXPERIMENT 1: EFFECTS OF DRUG VEHICLE (VEH), THE DRUGS CLORGYLINE (CL) AND DEPRENYL (D), *XENOPUS* SKIN MUCUS (SM), AND SM GIVEN WITH EACH DRUG (CL-SM AND D-SM) ON MEAN NUMBER OF NORMAL TONGUE FLICKS (T) AND THE MEAN MINUTES SNAKES CLIMBED (C) AND WERE ACTIVE (A)

	Clorgyline				L-Deprenyl						
Behavior	VEH	SM	CL	CL-SM	VEH	SM	D	D-SM	SE*	F†	*p*
T (#)	516.2^{a}	132.7^{b}	470.0^{a}	128.7^{bc}	515.2^{a}	215.5^{b}	611.1^{a}	22.2^{c}	225.1	13.90	0.0001
A (minutes)	12.7^{ab}	9.7^{bc}	13.7^{abc}	7.7^{c}	14.6^{ab}	9.0^{bc}	16.9^{a}	6.6^{c}	3.6	2.61	0.021
C (minutes)	7.1^{c}	18.3^{ab}	7.3^{c}	17.4^{ab}	5.4^{c}	14.9^{b}	14.7^{ab}	21.1^{a}	5.9	8.80	0.0001

Means with the same letter are not significantly different using LSD on square root transformed values; $\alpha = 0.05$.

*One SE for the mean calculated by taking the square root of the error mean squared divided by 12 (*n*) from the ANOVA.

†*F*-test for treatment effects from the ANOVA on square root transformed values using treatment × snake (order) as the error.

TABLE 4

RESULTS OF EXPERIMENT 2: EFFECTS OF 0, 5, 10, 20 μg/g CLORGYLINE AND L-DEPRENYL GIVEN IP IN COMBINATION WITH ORALLY APPLIED SHAM MUCIN OR *XENOPUS* SKIN MUCUS ON MEAN NUMBER OF NORMAL SNAKE TONGUE FLICKS (TF) AND MEAN MINUTES SNAKES CLIMBED (CLM) AND WERE ACTIVE (ACT) DURING 30-min SESSIONS

	Clorgyline				L-Deprenyl			
	Sham Mucin		*Xenopus* Mucus		Sham Mucin		*Xenopus* Mucus	
Behavior (μg/dose)	Mean*	SE (Diff)†	Mean	SE (Diff)	Mean	SE (Diff)	Mean	SE (Diff)
TF (#)								
0 →	562	100	373	100	784	86 --‡--	542	86
5	309	146	204	85	350	125‡	337	73
10	296	127	342	80	400	109‡	248	68§
20	269	118	92	65§	218	101§	18	55§
0 ←	278	86	297	86	249	86	154	86
5	364	125	170	73	373	125	46	73
10	220	109	200	68	264	109	113	68
20	521	101	336	55	190	101	126	55
ACT (minutes)								
0 →	14.1	3.8	12.1	3.8	17.6	3.3	14.6	3.3
5	15.1	5.5	8.0	7.3	23.0	4.7	14.0	6.2
10	12.8	9.1	14.0	4.4	19.3	7.8	11.8	3.8
20	13.6	8.6	9.6	6.1	16.1	7.4	3.7	5.3‡
0 ←	22.4	3.3 --§--	11.5	3.3	18.6	3.3 --‡--	10.9	3.3
5	22.9	4.7	11.8	6.2	17.7	4.7	8.9	6.2
10	25.8	7.8	10.8	3.8	13.9	7.8	8.0	3.8
20	20.0	7.4	10.1	5.3	5.3	7.4‡	6.8	5.3
CLM (minutes)								
0 →	5.8	2.4 --§--	16.2	2.4	5.5	2.0 --§--	20.5	2.0
5	8.3	2.5	25.1	2.1§	12.6	2.1‡	23.4	1.8
10	5.2	3.1	20.6	1.8	17.6	2.7§	26.8	1.5§
20	2.9	3.2	21.3	4.4	21.5	2.8§	29.5	3.7
0 ←	11.6	2.0 --§--	22.1	2.0	19.0	2.0 --§--	27.2	2.0
5	8.9	2.1	24.7	1.8	14.4	2.1	24.6	1.8
10	6.7	2.7	22.8	1.5	19.3	2.7	26.8	1.5
20	11.4	2.8	17.4	3.7	25.6	2.8	18.4	3.7

Drugs were given in increasing (→) and decreasing (←) dosage orders.

*The mean of each dose was compared to the mean of the zero dose using the least squares mean (‡$p < 0.05$, §$p < 0.01$) Bonferroni's multiple-comparison procedure was used to compare each set of three doses to its control.

†SE (Diff) is the SE of the difference between the dose mean and the zero dose mean. SE for the zero dose is the SE of the difference between the vehicle means for sham mucin and *Xenopus* mucus.

activity attenuated below the vehicle response rates. *Xenopus* mucus given with vehicle had no significant effect on activity.

DISCUSSION

Our results indicate that inhibition of MAO-A by clorgyline or of MAO-B by L-deprenyl induces no orofacial dyskinesias in snakes when these drugs are given with sham mucus (porcine mucin). However, when the highest doses (20 μg/g) were administered with *Xenopus* mucus clorgyline and deprenyl significantly attenuated mucus-induced yawning in adult and juvenile snakes, respectively. No other mucus-induced oral dyskinesias (fixed yawning, gaping, fixed gaping, chewing, writhing tongue) were modulated by MAO inhibition. In mammals (28), high doses of clorgyline or deprenyl result in the loss of substrate specificity. Therefore, it is possible that the attenuation of mucus-induced yawning by the highest dose of each drug resulted in the inhibition of both MAO-A and -B. If mucus-induced yawning is related to neuroleptic actions of the mucus (1,2), then the more complete inhibition of MAO could add to the available synaptic pool of dopamine, a consequence that might attenuate mucus-induced yawning.

The differential effect on yawning by clorgyline only in adult snakes and by deprenyl only in juvenile snakes suggests that the importance of MAO-A or -B varies with age or that drug sensitivity changes. But, as MAO-B inhibition by deprenyl attenuated tongue flicking, induced climbing, and potentiated mucus-induced climbing in juvenile (all doses) and adult snakes (only high dose used), these behavioral effects of deprenyl were probably not age dependent.

Both clorgyline and deprenyl are irreversible MAO inhibitors. By employing increasing and decreasing sequences of doses for clorgyline and deprenyl in EXP2, an assessment could be made of drug carryover effects between weekly treatments. MAO-B inhibition by deprenyl produced an obvious cumulative effect on climbing and tongue flicking. For example, when 20 μg deprenyl was administered first, it induced a potent climbing response that continued even when the drug vehicle was tested last, yet deprenyl-induced climbing in-

creased significantly with increasing doses. These results suggest that MAO-B inhibition by deprenyl induces effects in snakes that last at least 7 days. Prolonged inhibition by deprenyl also has been demonstrated in studies involving humans and laboratory mammals (11,18,27,32). As clorgyline had little effect on climbing, activity, and tongue flicking when compared to deprenyl, carryover inhibition between weekly doses was not apparent.

Previously, it was hypothesized that the stereotyped climbing response of snakes to oral administration of *Xenopus* skin mucus was a snake's behavioral mechanism for counteracting mucus-induced hypertension (1). By climbing vertically and leaving the water, gravity would pool blood caudally and reduce blood pressure in the head and other vital organs. That hypothesis was founded in part upon the cardiovascular physiology of arboreal snakes and their response to vertical positions and gravity (19,20). Vertical positions are accompanied by cardiovascular facilitative movements (CFMs) of the body that proceed cranially and are independent of locomotor muscular contractions. CFMs augment the flow of blood to the heart and brain (20) and can be induced by administering hypotensive drugs (19). In our study and other experiments (1-3,38), CFMs were never observed during the vertical climbing of water snakes administered *Xenopus* mucus. Thus, climbing induced by *Xenopus* mucus may counteract the hypertensogenic properties of the mucus.

The present study unexpectedly revealed significant climbing induced by all doses of deprenyl given with the porcine mucin and potentiation by deprenyl of mucus-induced climbing. Effects on blood pressure were expected only from clorgyline because inhibition of MAO-A is associated with hypertensive crises (cheese effect in conjunction with dietary amines) and conserved norepinephrine and 5-HT, both of which have hypertensogenic properties in peripheral circulation. Clorgyline given IP or ICV to spontaneously hypertensive rats decreased blood pressure possibly by elevating brain norepinephrine, which in turn inhibits sympathetic ganglia that regulate blood pressure; deprenyl had no effect on blood pressure (8,33). Yet, regardless of whether we administered clorgyline with porcine mucin or *Xenopus* mucus clorgyline had little effect on climbing. Human studies involving deprenyl and dietary tyramine have shown that >20-mg doses of deprenyl increased blood pressure (28). This suggested that deprenyl loses its MAO-B specificity at doses >20 mg. Others have also reported the loss of specificity of high doses of deprenyl (7,13). We suggest from our study that the loss of specificity of deprenyl at high doses is unlikely because clorgyline had no significant effect on climbing in both EXPs, while deprenyl consistently induced climbing at all doses tested on juvenile and adult snakes.

A valid question exists as to whether deprenyl itself was responsible for inducing climbing, attenuating normal tongue flicking, and potentiating these same *Xenopus* mucus-induced behaviors. For example, methamphetamine and amphetamine are metabolites of deprenyl (26,35), and amphetamines may cause the release of dopamine and norepinephrine, which could then lie more proximate to the behavioral responses observed (14). However, others have suggested that the phenylethylamine (PEA) that accumulates upon administration of deprenyl in mammals is more capable of releasing neuronal dopamine and norepinephrine than amphetamine (14). It has also been proposed that deprenyl acts indirectly by increasing the neuronal concentration of PEA, which then potentiates dopaminergic transmission (25). Another factor that links PEA to behaviors we observed is the well-documented increases in locomotor activity and stereotyped behaviors induced in rats by PEA (23,24). These stereotypies, however, are different from those caused by amphetamine, and the PEA stereotypy potentiated by deprenyl is probably not associated with the amphetamine metabolite because other MAO-B inhibitors that are not metabolized into amphetamine also potentiate PEA stereotypy (24). Thus, we suspect that PEA, a substrate of deprenyl, is more important in our study than the amphetamine metabolites of deprenyl.

Three studies from our laboratory now suggest that the snake's dopaminergic system is one important target of the mucus. For example, blockade of D1 (2) or D2 (1) dopamine receptors, using SCH23390 and haloperidol, respectively, potentiated mucus-induced yawning and attenuated fixed gaping. However, D1 blockade selectively potentiated writhing tongue movements and attenuated chewing movements while D2 blockade potentiated chewing movements. Unlike haloperidol, SCH23390 given alone attenuated activity and normal tongue flicking, but when given with *Xenopus* mucus SCH23390 increased activity and tongue flicking to normal control levels. Neither drug modulated the climbing induced by frog mucus nor induced climbing when administered with porcine mucin. We suggest that dopamine and/or PEA, the substrates for MAO-B, promote mucus-induced climbing and normal tongue flicking but may have some protective role against mucus-induced yawning. As D1 and D2 blockade potentiates mucus-induced yawning (1,2), it is not surprising that the inhibition of dopamine metabolism by high doses of deprenyl or clorgyline would attenuate the yawning induced by *Xenopus* mucus. Dopamine and/or PEA, in particular, appear involved in mucus-induced climbing because only MAO-B inhibition induced climbing. If climbing is a response to mucus-induced hypertension (1) and MAO-B substrates are involved, then dopamine receptors in peripheral vasculature and the heart may be sites of action for agents in mucus and for the MAO-B inhibitor deprenyl. Unfortunately, it is not known whether snakes differ from mammals in their organ compartmentalization of MAO-A and -B (36) and in their distribution and responsiveness of peripheral dopamine receptors (7). It is curious, however, that MAO-B is the most abundant form of MAO in the mammalian adrenal glands (36) and that the species of snakes with the largest adrenal glands are those that eat frogs and toads (29,30). Observations from our laboratory indicate that *Xenopus* mucus induces a profound dystonia and is lethal when administered to mammaphagous snakes such as black rat and corn snakes. Frog-eating snakes such as *Nerodia* (1-3), *Lycondonomorphus* (38), and *Heterodon* (unpublished results) exhibit the milder orofacial dyskinesias and climbing behaviors reported here.

Based upon the data at hand, we are testing the hypothesis that tolerance of frog-eating snakes and hypersensitivity of mammal-eating snakes to *Xenopus* mucus is linked to PEA and to specific organs and the ratio of MAO-B to -A they may contain.

ACKNOWLEDGEMENTS

The authors thank Dr. Marcia L. Gumpertz (NCSU Statistics) for statistical consultations, Faye L. Childers and Joy M. Smith (Applications Analysts, NCSU Statistics) for running data analyses, and Drs. John G. Vandenbergh and Robert Grossfeld (NCSU Zoology) for reviewing the manuscript. This work was supported in part by the North Carolina Agricultural Research Service (Raleigh, NC).

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