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SCH 23390: D-1 Modulation of Oral Dyskinesias Induced in Snakes by *Xenopus* Skin Mucus

GEORGE T. BARTHALMUS

Department of Zoology, North Carolina State University
Raleigh, NC 27695-7617

AND

KATHERINE B. MEADOWS

Undergraduate Honors Program, Department of Zoology
College of Agriculture and Life Sciences, North Carolina State University
Raleigh, NC 27695–7642

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BARTHALMUS, G. T. AND K. B. MEADOWS. *SCH 23390: D-1 modulation of oral dyskinesias induced in snakes by* Xenopus *skin mucus*. PHARMACOL BIOCHEM BEHAV **36**(4) 843–846, 1990.—The granular gland skin secretion of *Xenopus laevis* induces seven involuntary oral dyskinesias and climbing behavior in the water snake *Nerodia sipedon*. In a previous study the D-2 receptor antagonist, haloperidol (HAL), selectively potentiated mucus-induced yawning and chewing but attenuated fixed gaping; other oral behaviors were unaffected; HAL alone induced no dyskinesias and failed to modify mucus-induced decreases in tongue flicking, cage climbing and activity. As skin compounds have neuroleptic properties known to induce human and animal dykinesias, we hypothesized that D-1 receptor antagonism may modulate the four of seven mucus-induced dyskinesias and the climbing not altered by HAL. We found that, like HAL, SCH 23390 (SCH) potentiated mucus-induced yawning, attenuated fixed gaping and had no effect on climbing. Unlike HAL's potentiation of chewing, SCH attenuated chewing and potentiated writhing tongue movements. SCH alone, like skin mucus, attenuated tongue flicking and activity but, given with mucus, SCH increased tongue flicking and activity to control levels. Compared to the HAL study, results suggest that mucus-induced yawning and fixed gaping are similarly modulated by both HAL and SCH, while these drugs have opposite effects on writhing tongue and chewing. SCH given alone or with frog mucus had unique effects on activity and normal tongue flicking.

Xenopus skin mucus Yawning *Nerodia* Dyskinesia Dopamine Neuroleptic SCH 23390 D-1 D-2 Movement disorders

AN antipredatory strategy of the African clawed frog, *Xenopus laevis*, appears to involve compounds from the granular skin glands that induce involuntary orofacial dyskinesias and climbing behavior in American (2,3) and African water snakes (17). The skin's mucus contains biogenic peptides (caerulein, cholecystokinin octapeptide, thyrotropin-releasing hormone, xenopsin) and indoleamines (serotonin, bufotenidine) with known neuroleptic properties [see (3)]. As most drug-induced orofacial dyskinesia and dystonia occurs in schizophrenics treated chronically with D-2 dopamine receptor blockers (15), an earlier study from our laboratory (2) tested the hypothesis that haloperidol (HAL), given prior to oral application of *Xenopus* skin mucus, would potentiate the mucus-induced dyskinesias and cage climbing in the American water snake, *Nerodia sipedon*. That study reported seven mucus-induced involunatry orofacial behaviors, however, HAL selectively potentiated only the yawning and chewing behaviors but attenuated fixed gaping. HAL given alone failed to modify the induced oral behaviors, cage climbing, spontaneous locomotor activity and normal tongue flicking. Skin mucus given alone reduced locomotor activity and normal tongue flicking. Because of HAL's selective modulation of only three of the seven mucus-induced dyskinesias, and because both the 0.05 and 0.5 μg/g doses caused identical effects on mucus-induced behaviors, we tested the hypothesis that D-1 receptors may govern some or all of the orofacial behaviors not affected by the D-2 blocker, HAL. Here we report that the selective D-1 receptor blocker SCH 23390 (SCH) has properties that are both unique and common to the D-2 modulating actions of HAL on mucus-induced behaviors in *Nerodia*.

METHOD

The subjects (N = 6) were drug-naive 20-month-old sibling *N.*

TABLE 1

MEAN OROFACIAL BEHAVIORS OF 3 40-MINUTE SESSIONS WHERE *XENOPUS* SKIN MUCUS (M) WAS GIVEN ORALLY (TO 6 SNAKES) ALONE OR TOGETHER WITH 0.05, 0.5 OR 10 μg/g BODY WEIGHT SCH 23390

Behavior	M+Veh	M+0.05 SCH	M+0.5 SCH	M+10 SCH	1 S.E.*	F†	$p>F$
Yawning‡	18.9[a]¶	17.8[a]	24.6[a]	43.5[b]	0.3/0.3	10.0	0.0009§
Fixed Gaping	6.9[a]	7.2[a]	4.6[b]	2.7[b]	0.8/0.9	4.63	0.0188§
Wr. Tongue‡	2.3[a]	3.2[a]	4.5[b]	9.1[b]	0.2/0.3	6.11	0.0071§
Fixed Yawning‡	1.7	1.1	1.5	1.7	0.2/0.2	0.45	0.7226
Gaping	29.1	31.1	24.2	26.1	6.4/7.8	0.32	0.8118
Chewing‡	10.4	5.3	6.9	5.3	0.3/0.3	2.32	0.1193
Mean Totals	69.3	65.7	66.3	88.8			

*Standard error of a mean calculated using the Snake·Drug mean-square from the ANOVA. The first number is for 6 snakes and the veh., 0.05 and 0.5 doses; the second number is for 5 snakes (one died) at the 10 μg dose.

†ANOVA F-test for no difference between the four drug doses.

‡Analysis is performed on the square root-transformed data.

§Highly significant mucus-SCH 23390 interaction.

¶Within a row, means without a letter in common differ significantly ($\alpha = 0.05$) using the protected LSD procedure and Snake·Drug as the error.

sipedon born in the laboratory during July 1987. They were littermates of snakes tested with HAL in an earlier study (2). Initial body weights (29–57 g) increased (40–63 g) during the 98-day investigation. Experimental protocol was identical to that of the earlier HAL study (2). Briefly, snakes were reared individually, fed fish to repletion once each week, and were maintained on a photoperiod of 14 L:10 D at ambient laboratory temperatures. Male *Xenopus* (4–5 cm body length) served as donors of skin mucus [see (2) for other details].

Procedure

Trials occurred in 40-l aquaria containing 3 cm of tap water at 26°C. Each of two observers was assigned 3 snakes; Observer No. 1 tested snakes A, C and E, and Observer No. 2 examined snakes B, D and F. Two snakes, e.g., A with B, C with D, and E with F were tested as a pair but within separate enclosures; a single frog served as a mucus donor for testing one pair of snakes. In this way, differences could be established between two snakes exposed to the mucus from the same frog.

Snakes were first administered SCH 23390 (Schering Corp.) or drug vehicle (0.02 M tartaric acid in water) and observed for 20 minutes. Then, either frog mucus or porcine mucin (Sigma Chemical), a control for the physical presence of mucus in the mouth, was applied to the roof of a snake's mouth and behavior was recorded for an additional 40 minutes. SCH at 0.05, 0.5 or 10.0 μg/g body weight, or the drug vehicle was delivered ventro-laterally into the posterior coelom as 0.05 cc per 5 g body weight. The white skin mucus was collected by injecting 0.012 mg epinephrine, dissolved in 0.2 cc distilled, deionized water, into the dorsal lymph sac of *Xenopus*. To prevent rapid drying and thickening of the mucus, water 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. The porcine mucin (sham control) was mixed with tap water to a consistency similar to that of the frog mucus.

Three replicate trials for each of 8 treatments were performed on 6 snakes. Replicate determinations were performed every 3–4 days. The treatment order was: vehicle and sham mucin; vehicle and frog mucus; 0.05 μg/g SCH and sham mucin; 0.05 μg/g SCH and frog mucus; 0.5 μg/g SCH and sham mucin; 0.5 μg/g SCH and frog mucus; 10 μg/g SCH and sham mucin; 10 μg/g SCH and frog mucus.

Behaviors Recorded

Oral behaviors observed included *Gaping* (G): slight opening then closing of the mouth; *Yawning* (Y): wide prolonged opening then closing of the mouth, often with the head dorsoflexed; *Fixed Gaping* (FG): gaping longer than 4 seconds; *Fixed Yawning* (FY): yawning longer than 4 seconds; *Tongue Flicking* (T): normal, rapid protrusions of the tongue; *Writhing Tongue* (WT): prolonged writhing movements of the tongue; *Chewing* (CHEW): alternate raising and lowering of the right and left jaw maxilla; *Gulars* (GUL): the temporary expansion of the throat as in burping. Other behaviors recorded included aquarium wall climbing (in minutes) to <50 percent (15 cm) of the aquarium wall (LOWUP), and HIGHUP if >50 percent (15≥30 cm); and *Activity* (A): the time spent actively moving in the aquarium.

Statistical Analyses

A repeated measures ANOVA was used to test for "observer," mucus, drug, and mucus-drug effects. As porcine mucin induced no oral behaviors, Table 1 only presents frog mucus data where "drug" effects were calculated using the Snake by Drug mean square as the error. In Table 2, where data from both porcine mucin and frog mucus (with or without drug) are presented, the effects of frog mucus, drug, and the mucus-drug combination were calculated using the Snake by Treatment mean square as the error. Pairwise comparisons of means were conducted only if the appropriate ANOVA F-test was significant using the protected least significant difference procedure and Snake by Treatment as the error.

RESULTS

Very few involuntary oral behaviors (G, FG, Y, FY, CHEW, WT) were observed following the administration of porcine mucin with drug vehicle or when porcine mucin was paired with 0.05,

TABLE 2

MEAN NUMBER OF NORMAL TONGUE FLICKS (T), MINUTES ACTIVE (A) AND MINUTES LOW CLIMBING (LC), HIGH CLIMBING (HC) AND TOTAL CLIMBING (C) PER 40-MINUTE SESSION WHERE FROG MUCUS (M) OR PORCINE MUCIN (PM) WAS GIVEN ORALLY ALONE OR TOGETHER WITH 0.05, 0.5, or 10.0 μg/g BODY WEIGHT SCH 23390

Behavior	PM+VEH	PM+ 0.05	PM+ 0.5	PM+ 10.0	M+VEH	M+0.05	M+0.5	M+10.0	1 SE*	MF	SCHF	MSCHF
T (No.)	355.5[a]‡	190.3[b]	222.0[b]	190.5[b]	182.4[b]	217.7[b]	270.3[a]	275.6[a]	32/35	0.09	1.49	6.33†
A (min)§	12.5[a]	7.2[b]	7.9[b]	7.7[b]	6.7[b]	7.4[b]	9.3[a]	9.8[a]	0.2/0.2	1.15	1.95	4.92†
LC (min)§	5.7	3.1	1.9	6.5	11.9	14.8	15.7	12.8	0.3/0.4	71.9†	0.25	1.45
HC (min)§	1.5	1.1	0.7	0.3	12.4	10.9	9.8	8.7	0.4/0.4	63.6†	0.29	0.02
C (min)§	7.2	4.2	2.6	6.8	24.3	25.8	25.0	21.5	0.4/0.4	141.7†	0.55	0.77

*Standard error of the mean using the Snake·Treatment mean square from the ANOVA; the first number is for 6 snakes and the veh., 0.05 and 0.5 doses; the second number is for 5 snakes at the 10.0 μg dose.

†HIghly significant frog mucus (M) effect ($p<0.0001$) or mucus-drug effect ($p<0.001$) using Snake·Treatment as the error where Treatment represents all 8 mucus-drug combinations.

‡Within a row, means without a letter in common in parentheses differ significantly (alpha = 0.05) using the protected LSD procedure and Snake·Treatment as the error.

§Analysis is performed on square root-transformed data.

MF = F-value for the skin mucus effect; SCHF = F value for the drug effect; MSCHF = F value for the mucus-drug effect.

0.5 or 10 μg/g SCH; so, those data were included neither in the ANOVA nor in Table 1. However, all treatments involving frog mucus, with or without SCH-induced orofacial behaviors (Table 1) and altered T, A, LC, HC, and C (Table 2) were analysed. Here the repeated measures ANOVA was applied to all data and tests for drug and mucus main effects and a drug by mucus interaction were performed. Observer effects were noted only for GUL so those data are not presented. One snake died during trials with the 10 μg SCH, so data from five rather than six snakes is reported (with corresponding standard errors) in Tables 1 and 2 for that dose.

The 10 μg dose of SCH significantly potentiated mucus-induced Y, however, the 0.5 and 10 μg doses significantly attenuated FG (Table 1). WT was potentiated by the 0.5 and 10 μg doses. Thus, three of the six mucus-induced oral dyskinesias were modulated by the D-1 dopamine receptor blocker SCH 23390. Table 1 also reveals that the mean totals of mucus-induced oral behaviors involving vehicle, and the 0.05 and 0.5 μg doses were nearly identical, however, the 10 μg dose increased the mean total by over 30%, most of which arose from increased Y and WT.

Minutes spent LC, HC and C (Table 2) were significantly increased by oral application of frog mucus, however, SCH had no effect on these climbing behaviors when given with porcine mucin or frog mucus.

Frog mucus given with drug vehicle (M+Veh) significantly lowered T and A from control levels (PM+Veh) of responding. However, each dose of SCH given with porcine mucin significantly and similarly attentuated both T and A when compared to vehicle controls. SCH at 0.5 and 10 μg/g and given with frog mucus returned both T and A to the control (PM+Veh) baseline.

DISCUSSION

We have shown that the D-1 receptor antagonist SCH 23390 modulates orofacial behaviors (Y, FG, WT) induced in water snakes by *Xenopus* skin mucus, and that no orofacial behaviors appear when SCH is given alone or with porcine mucin. Mucus-induced climbing was not modulated by SCH but the 0.5 and 10 μg doses returned T and A to the high control (PM+Veh) rates of responding. When given with porcine mucin, SCH significantly attenuated T and A but without differences between doses.

In an earlier study (2) the D-2 antagonist haloperidol (HAL) at 0.05 and 0.5 μg/g dramatically potentiated Y and CHEW but attenuated FG. In our study, only 10 μg SCH potentiated Y and attenuated FG, while CHEW decreased but not significantly by any dose. Although 0.05 and 0.5 μg HAL failed to modulate WT, SCH significantly potentiated WT but, again, only at the 10 μg dose. Further, HAL had no effect on T, C or A when given with either porcine mucin or frog mucus. SCH given with porcine mucin attenuated T and A but, when given with frog mucus, SCH returned both T and A to the high control rates of responding. Thus, frog mucus affected locomotor activity and normal tongue flicking in a pattern similar to that of D-1 but not D-2 antagonism, and only D-1 antagonism counteracted the attenuating effects of frog mucus on these behaviors (an apparent reverse interaction).

As in the HAL study, the mean total induced oral behaviors (Table 1) seen when mucus was interacted with drug, was very stable (i.e., 69.3 M+Veh; 65.7 M+0.05; 66.3 M+0.5), although 10 μg SCH increased behavior (88.8 M+10) by >30%, a dose not administered in the HAL study. Possibly a similar effect would have been noted had 10 μg HAL been given in that study.

In comparing data between the HAL study (2) and this study, we observed that the 14-month-old snakes (HAL study) differed significantly from drug-naive 20-month-old siblings of this study. Age-related differences were examined by comparing the PM+Veh as well as M+Veh treatments between the HAL and SCH studies. Purely spontaneous behavior would be expected from snakes treated with PM+Veh, while only the action of frog mucus would be expected to modulate behaviors of snakes treated with M+Veh. In the PM+Veh treatment, although older snakes (this study) were 40% less active (A) than younger snakes, the rate of normal tongue flicking (T) was nearly the same. A profound age-related difference in these parameters was seen when the M+Veh treatments were compared. Older snakes elicited only 61% of the T and only 47% of the A elicited by younger snakes. Further, comparisons of ages revealed that the total mucus-induced oral behaviors of older snakes was only 59% of that seen in younger snakes. These differences are only quantitative and not qualitative because snakes of both ages revealed identical mucus-induced behaviors. As all snakes were born of the same mother and tested with an identical experimental protocol, we suspect that this model may provide an additional parallel with mammalian studies that also reveal age-related differences in susceptibility to movement disorders [see (14,15) for review]. However, mucus-induced climbing (C) behavior was similar between both age groups. Although

apomorphine-induced C (9) and yawning (10) in rodents have been shown to decrease with administration of SCH, we have seen no such antagonism on these mucus-induced behaviors in snakes. We suspect that this observation further supports evidence stated in the HAL study (2) that mucus-induced C is governed by the hypertensogenic properties of the frog mucus and not by the neuroleptic properties.

Some have suggested that SCH 23390 has neuroleptic properties and, therefore, it may induce oral dyskinesias typically induced by D-2 receptor blockers [see (7) for review]. Indeed, we have shown that SCH has effects on snakes administered frog mucus that are identical to those modulated by HAL [e.g., potentiated Y; attenuated FG (2)]. However, SCH exhibited properties unique from HAL in that SCH potentiated rather than attenuated WT, and CHEW tended to decrease with SCH rather than to significantly increase as with HAL.

Studies have reported that SCH blocks D-2 agonist-induced Y in rodents (8,13), however, in snakes both HAL (2) and SCH potentiated mucus-induced Y. At least two possibilities may account for these observations. First, as Y in rodents is induced by minute amounts of D-2 dopamine agonists and is a response that is blocked by SCH, it may be speculated that *Xenopus* mucus is not acting as a D-2 agonist because SCH potentiated mucus-induced Y in snakes. However, D-1 and D-2 receptors may be linked in a way that the blockade of one results in the inactivation of the other (13). Second, it has been reported that SCH acts upon D-1 as well as 5-HT_1 (12) and 5-HT_2 receptors (5,6). Thus, the similar actions of HAL and SCH may be related to their neuroleptic properties, while the unique effects of SCH in modulating mucus-induced behaviors may involve actions on one or more 5-HT receptor subtypes, particularly in light of the abundance of 5-HT and bufotenidine (1,4) in *Xenopus* skin mucus.

Compounds in *Xenopus* skin mucus induce identical and highly stereotyped orofacial dyskinesias in an American water snake (2,3) and African snake predators known to eat *Xenopus* (17), although the latter are less sensitive to the mucus. Numerous studies have shown that compounds abundant in *Xenopus* skin mucus, such as cholecystokinin octapeptide and caerulein, are linked to dopaminergic involvement in tardive dyskinesia and schizophrenia (16). Because D-1 and D-2 receptor blockers modulate mucus-induced oral behaviors in ways similar to their modulation of dopamine agonist-induced oral behaviors (7,15), we propose that this snake-*Xenopus*-mucus paradigm is a natural and unique behavioral model for studying movement disorders by assessing the actions of D-1, D-2 and 5-HT receptor agonists and antagonists. The mucus-induced oral dyskinetic behaviors reported here closely parallel those observed in neuroleptic-induced human and animal movement disorders [see (2)] and, more recently, the report of neuroleptic-induced "persistent open mouth" (11), a human behavior that resembles the fixed yawning and fixed gaping seen in mucus-treated snakes (2, 3, 16). As government agencies and the private sector seek alternatives to mammalian models for drug assessment, this naturally occurring model deserves further development.

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