Pharmacology Biochemistry & Behavior, Vol. 30, pp. 957–959. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Xenopus Skin Mucus Induces Oral Dyskinesias That Promote Escape From Snakes

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Received 9 November 1987

BARTHALMUS, G. T. AND W. J. ZIELINSKI. Xenopus *skin mucus induces oral dyskinesias that promote escape from snakes*. PHARMACOL BIOCHEM BEHAV **30**(4) 957–959, 1988.—African clawed frogs fed to American water snakes induced yawning and gaping which slowed ingestion and facilitated the frogs' escape without inducing flavor aversion. The peptide and/or indolealkylamine contents of the frog's poison glands caused the effect because frogs with purged glands did not induce these behaviors and rarely escaped. Poison gland mucus, applied orally, elicited similar oral movements. The frog's clear lubricating mucus was inactive. As several compounds in the poison glands have known neuroleptic properties, the oral behaviors may be induced by neural mechanisms reported to govern neuroleptic-induced orofacial dyskinesia in schizophrenics.

Xenopus Amphibian skin peptides Yawning *Nerodia* Tardive dyskinesia Neuroleptics CCK-8
Caerulein Xenopsin TRH Serotonin Antipredation Movement disorders

THE skin secretions of many amphibians contain peptides that are identical to, or close analogues of, peptides found in the vertebrate brain and gut [10]. Despite studies on peptides that form this "brain-gut-skin triangle" [7], little is known of their role within amphibian skin. As poison glands of the African clawed frog *(Xenopus laevis)* contain only peptides and indolealkylamines [1, 3, 9, 12], we explored the potential antipredatory role of these compounds by feeding *Xenopus* to American northern water snakes *(Nerodia sipedon)*. Here we report that such feedings cause dyskinetic yawning and gaping movements in the snake that frequently permitted a frog's escape. The dyskinesia is predictable given the known actions of each skin compound and their coincidence with neural mechanisms believed to underly drug- and peptide-induced oral dyskinesias in mammals including man.

METHOD

Snakes captured in Wake County, NC were reared individually in 40-l dry aquaria, and fed minnows once weekly prior to this study. The body weight of 11 adult snakes ranged from 75–299 g. Clawed frogs (Xenopus I, Ann Arbor, MI) were reared in a flow-through tank at 18°C and 12:12 photoperiod and were fed pelleted food. Two experiments were conducted: one where snakes were presented live frogs, and a second where extracted skin mucus was applied to the snakes' mouths.

Experiment 1

Snakes were offered one male frog (5% of the snake's weight) every 7 days for 10 weeks. Eleven snakes were each offered 5 frogs with full poison glands (toxin-loaded=TL) and 5 frogs whose glands were emptied (toxin-free=TF) by injecting 0.012 mg epinephrine, dissolved in 0.2 cc tap water, into the dorsal lymph sac. The white mucus that appeared on the skin was removed with towels immediately and again 4 hr later. A 4-hour delay before testing was sufficient for recovery from effects of epinephrine but was an insufficient period for poison glands to refill [3]. Trials occurred in 40-l aquaria containing 10 cm of tap water. Five snakes received 5 TL frogs followed by 5 TF frogs (Sequence 1) and 6 snakes were fed in the reverse order (Sequence 2). Data recorded included time between first capture and consumption (down-time), pre- and postingestive oral behaviors, and frog escapes and recaptures. Oral behaviors included: *Gaping*—slight opening then closing of the mouth; *Yawning*—wide prolonged opening of the mouth often with the head dorsoflexed; *Writhing tongue*—prolonged writhing movements of the tongue. Sessions ended 1 hour after any of these criteria: (a) disappearance of the frog in a snake's mouth; (b) failure of a snake to recapture and escaped frog; (c) a fifth escape; or (d) no attempt to feed. An escape was recorded when a frog struggled and escaped or when a snake released

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TABLE 1

MEAN (± 1 S.E.) NUMBER OF FROG ESCAPES AND SNAKE BEHAVIORS PER SESSION FOR 11 SNAKES FED 5 TOXIN-LOADED (TL) AND 5 TOXIN-FREE (TF) FROGS

Treatment	Down-Time (min)	Escapes (No.)	Gapes (No.)	Yawns (No.)	Writhing Tongue (No.)
TL	37.6 ± 6.7	1.0 ± 0.2	17.3 ± 2.4	16.7 ± 2.8	0.3 ± 0.1
TF	12.6 ± 3.0	0.1 ± 0.2	2.3 ± 0.8	2.4 ± 1.4	0

a frog. The few snakes that did not eat always attempted to feed the next day.

A repeated measures ANOVA was used to test for Sequence and Treatment (TL or TF frogs) effects. Analyses were applied to log-transformed down-time and square root-transformed escape, yawn, gape and writhing-tongue data.

RESULTS

Treatment was a significant effect for all variables measured. Thus, TL frogs took longer to ingest, $F(1,9)=39.01$, $p=0.0002$, escaped more frequently from initial or successive captures, $F(1,9)=15.54$, $p=0.0001$, and caused more Yawns, $F(1,9)=5.50$, $p=0.0437$, Gapes, $F(1,9)=15.48$, $p=0.0034$, and Writhing Tongue Movements, $F(1,9)=7.14$, $p=0.0255$, than TF frogs (Table 1). Sequence was not a significant effect for any variable [Down-Time, $F(1,9)=0.06$, $p=0.8058$; Escapes, $F(1,9)=4.6$, $p=0.0726$; Gapes, $F(1,9)=0.47$, $p=0.5115$; Yawns, $F(1,9)=3.28$, $p=0.1037$; Writhing Tongue Movements, $F(1,9)=0.04$, $p=0.8402$] nor was the Sequence by Treatment interaction [Down-Time, $F(1,9)=0.21$, $p=0.6554$; Escapes, $F(1,9)=1.76$, $p=0.3001$; Gapes, $F(1,9)=0.65$, $p=0.4407$; Yawns, $F(1,9)=1.89$, $p=0.2019$; Writhing Tongue Movements, $F(1,9)=0.04$, $p=0.8402$].

All snakes attacked or attempted to consume TL frogs. Also, each snake usually attempted to recapture an escaped TL frog suggesting that appetite was unaffected and that TL frogs were not flavor aversive.

Experiment 2

To confirm that skin secretions caused the antipredatory effects, four substances were applied to the snake's palate; (1) the white, viscous, poison gland mucus exuded after epinephrine injection, (2) porcine stomach mucin (Sigma Chemical), to control for the physical presence of mucus in the mouth, (3) porcine mucin mixed with 0.012 mg epinephrine, to control for epinephrine that may have leaked through the skin and was applied with frog mucus, and (4) 0.4 cc of clear, lubricating frog mucus collected from uninjected frogs. Treatments were applied to 7 snakes fed frogs 60 days earlier. Sessions began after the application of the test compound and oral behaviors were recorded for 30 minutes during 3 weekly trials. Treatments were alternated so that no snake received the same compound on successive trials.

RESULTS

Only toxic mucus consistently induced yawning and gaping (Table 2). Fixed yawns and gapes (lasting >4.0 sec) were common in toxic mucus trials. Snakes yawned and gaped usually after having climbed the tank walls. Some fixed yawns and gapes occurred under water suggesting that yawning and gaping need not be associated with breathing. We conclude that toxic mucus induced involuntary yawning and gaping and permitted frogs to escape because few oral behaviors appeared in snakes given other mucus treatments.

TABLE 2

MEAN (± 1 S.E.) NUMBER OF ORAL BEHAVIORS FOR 3, 30 MINUTE SESSIONS WHERE 7 SNAKES WERE TREATED WITH 4 DIFFERENT MUCUS APPLICATIONS

	Type of Mucus Applied			
Behavor	Toxic Mucus*	Porcine Mucin	Mucin Plus Epinephrine	Clear Mucus
Gape	21.1 ± 2.2	1.3 ± 0.4	0.9 ± 0.3	0
Yawn	19.0 ± 2.2	0.4 ± 0.3	0.3 ± 0.2	0
Gape + yawn	40.1 ± 3.6	1.8 ± 0.6	1.3 ± 0.5	0
Fixed gape	5.7 ± 1.2	0	0	0
Fixed yawn	7.3 ± 1.8	0	0	0
Writhing tongue	0.9 ± 0.7	0	0	0

*Toxic mucus effects were significantly greater than the other three mucus treatments for each dependent variable (Duncans Multiple Range Test; experiment-wise alpha=0.05).

DISCUSSION

Xenopus skin mucus contains the indolealkylamines, serotonin (5-HT) and bufotenidine (BF), and the following peptides: cholecystokinin octapeptide (CCK-8); caerulein (CRL), a close structural and functional analogue of CCK-8; thyrotropin-releasing hormone (TRH); and xenopsin (XN), an analogue of neurotensin [1, 3, 6, 9, 12, 16]. Curiously, the action of each compound is compatible with hypotheses governing the tardive dyskinesias (TD) seen in schizophrenics treated chronically with dopaminergic blockers (the neuroleptics) [13]. TD is an extrapyramidal dysfunction in which involuntary yawning, chewing and tongue movements occur. However, as dopamine inhibits release of tuberoinfundibular peptide hormones, the neuroleptics also elevate plasma levels of prolactin and oxytocin [2,15], alpha-melanocyte stimulating hormone and adrenocorticotropin [4,11], which have all induced yawning in lab animals. As CCK-8 and CRL [17,21] and 5-HT [8] have neuroleptic properties, and XN may possess the neuroleptic properties of neurotensin [5], we suspect that these *Xenopus* skin compounds mimic neuroleptics and create a neurochemistry for oral dyskinesia in snakes that promotes the escape of frogs.

Further, 5-HT and bufotenidine [19] and TRH [18] elevate serum prolactin levels and oxytocin, the most potent known inducer of yawning [2], is dramatically elevated in plasma of rats administered CCK [20].

Our observation that oral dyskinesias occur within 30 seconds of oral contact with mucus suggests that toxins are absorbed orally and/or react with oral receptors. The snake's vomeronasal organ (VNO), a contact receptor that mediates detection of prey and the rate of tongue flicking [14], is probably not the chief target for skin toxins because two snakes given VNO nerve transections (but unconfirmed histologically) exhibited all presurgical dyskinesias.

This new behavioral model of orofacial dyskinesia appears biomedically promising given that the known compounds in *Xenopus* skin occur naturally in the mammalian brain or occur as close analogues of neurochemicals. Whether skin agents act in combinations or alone is the focus of our future studies. We are now assessing the behavioral responses of *Lycodonomorphus rufulus*, a natural snake predator of *Xenopus* in South Africa (J. Visser, personal communication). Typically, sympatric predator and prey species coevolve adaptations to each other's defenses. Thus, if African water snakes prove unresponsive to *Xenopus'* skin mucus, and if this defense is neurologic, such as an unusual profile of neurotransmitters typically affected by neuroleptics, rapid advances in drug development can be made by comparing the brain chemistry of *Nerodia* with that of *Lycodonomorphus*.

ACKNOWLEDGEMENTS

We thank D. Woodward, N.C.S.U., Raleigh, who first observed similar behaviors in hognose snakes fed *Xenopus*, Dr. C. Brownie for statistical consultations, and Dr. M. Halpern, Downstate Medical Center, Brooklyn, for performing two VNO nerve transections on *Nerodia*. This work was supported by a grant from the NC Agricultural Research Service to G.T.B. and is paper No. 11091 of the Journal Series of the NC Agricultural Research Service, Raleigh, NC.

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