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Evidence for the bioactivation of slaframine

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SLAFRAMINE is an alkaloid which has been isolated from the fungus *Rhizoctonia leguminicola*.[1, 2] It was discovered as a result of its property of stimulating salivation in cattle consuming forages contaminated with fungus.[3, 4] In 1965 Rainey *et al.*[1] and Aust and Broquist[2] simultaneously reported the isolation of a crystalline salivation factor from pure cultures of the fungus. This crystalline factor was very potent in stimulating salivation; however, the compound showed little or no cholinergic action *in vitro*.[5] In 1966, Aust *et al.*[6] reported the structure of the salivation factor as 1-acetoxy-8-aminooctahydroindolizine and suggested the name slaframine (slafra, to slaver). Evidence is now presented for the conversion of slaframine to an active metabolite by the drug-metabolizing enzymes of the liver. These results would explain the lack of cholinergic activity *in vitro*.

Slaframine was isolated from pure cultures of *Rhizoctonia leguminicola*[2] and purified by repeated crystallization as the dipicrate and was then converted to the dicitrate for study. Purified 1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl)-ethane (DDT) and chlordane were supplied by Dr. L. L. Bieber of Michigan State University. Phenobarbital was obtained from Merck & Co., Rahway, N.J. 2-Diethylaminoethyl-2,2-diphenylvalerate · HCl (SKF-525A) and 2-ethylaminoethyl-2,2-diphenylvalerate · HCl (SKF-8742A) were supplied by Smith, Kline & French Laboratories, Philadelphia, Pa. 2,4-Dichloro-6-phenylphenoxyethylamine · HBr (DPEA) and 2,4-dichloro-6-phenylphenoxyethyldiethylamine · HBr (Lilly 18747) were supplied by Eli Lilly & Co., Indianapolis, Ind.

Quantitative salivation studies were made by cannulation of the salivary duct of anesthetized cats and recording the results by means of a photoelectric drop counter. Simultaneous records of heart rate, blood pressure and respiration were obtained. Quantitative pancreatic activity was determined by placing permanent cannulae in the pancreatic ducts of goats, sheep and calves. Cannula loops were inserted into the intestine for the purpose of returning the pancreatic fluid during the intervals when collections were not being taken. During collection periods, pancreatic fluid was collected for 30-min intervals to obtain an even average flow rate. Activation of slaframine *in vivo* was estimated by observing the time at which salivation started after the administration of slaframine, as judged by the formation of a drop of saliva on the mouth. For the induction of liver microsomal enzymes, some of the animals were pretreated with the inducers daily for 5 days prior to the day of testing. Inhibition experiments were conducted by giving the inhibitor 30 min prior to testing with slaframine.

Quantitative studies of salivary and pancreatic secretion demonstrated that there was always an appreciable induction period before the onset of activity after the administration of slaframine. Maximum salivary activity in anesthetized cats (Fig. 1) occurred about 1 hr after slaframine injections. This delay was appreciable compared to the instantaneous stimulation resulting from the administration of the cholinomimetic pilocarpine. It was also noted that the duration of salivation was extremely long, usually lasting for 6 hr after a single dose of 0·3 mg/kg (Fig. 1). In addition to salivation, it was noted that most of the exocrine glands were stimulated by slaframine. The ability of slaframine to stimulate specifically the exocrine glands was indicated by the fact that doses which elicited maximal secretory rates had no effect on heart rate, blood pressure or respiration. In addition, no effect of slaframine could be demonstrated on ganglionic transmission, peripheral blood flow or neuromuscular transmission. The administration of slaframine to animals with pancreatic cannulae also resulted in a substantial delay before an increase in pancreatic flow was observed (Fig. 2). This delay would be roughly correlated to the animals' ability to metabolize drugs. Goats are excellent metabolizers of xenobiotics and could activate slaframine much more readily than the calf, which is a poor metabolizer of xenobiotics[7] (Fig. 2).

Table 1 shows the relationship between the route of administration of slaframine and the delay before salivation was observed. In mice, it was demonstrated that a longer delay resulted after intravenous injection than with intraperitoneal administration. This delay could be further decrease by injections directly into the portal vein. The involvement of the liver was further investigated by

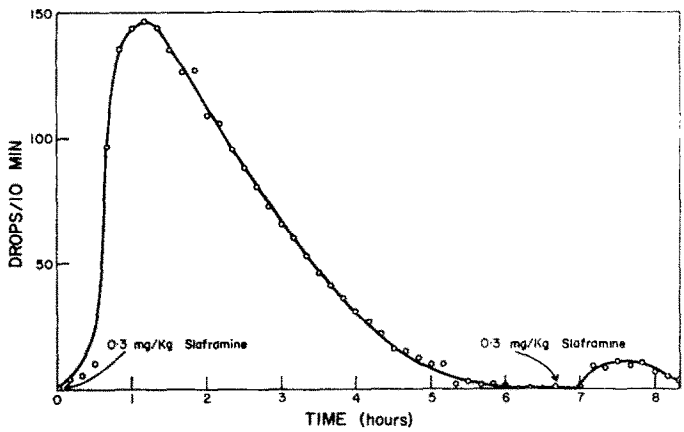

FIG. 1. Salivary activity (submaxillary) of an anesthetized cat after the administration of slaframine. The submaxillary duct of a pentobarbital anesthetized cat was cannulated and the rae of salivation in drops per 10 min was recorded by means of a photoelectric drop counter. Slaframine was administered by i.v. injections. Simultaneous recordings of blood pressure, heart rate and respiration rate were also obtained.

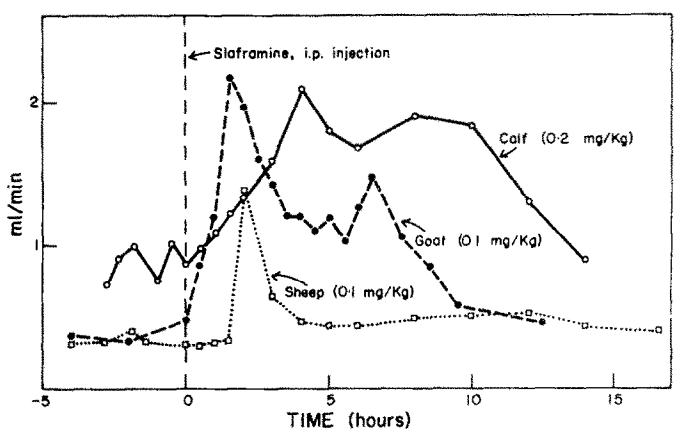

FIG. 2. Effect of slaframine on the pancreatic activity of various animals. The pancreatic duct was cannulated and the total volume of pancreatic fluid was collected and measured for 30-min intervals. After establishing a normal secretion rate, slaframine was administered intraperitoneally.

clamping the portal vein and hepatic artery of rats for various times while simultaneously injecting slaframine directly into the vena cava. It can be seen (Table 1) that the delay could be significantly extended by briefly isolating the liver and that it could be completely eliminated by the permanent isolation of the liver.

Further evidence that slaframine is transformed to its active metabolite by drug-metabolizing systems of the liver was obtained by the use of known inducers and inhibitors of these systems. An increase in the rate of activation by induction would result in a decreased delay in the onset of salivation after slaframine administration. Inhibition would result in the inverse, longer delays. Table 2

TABLE 1. EFFECT OF ROUTE OF ADMINISTRATION ON THE DELAY OF SALIVATION AFTER SLAFRAMINE ADMINISTRATION

Method and route of administration		Species	Dose (mg/kg)	Delay*
i.v. (tail vein)		mouse	2	24 ± 3·0
i.p.		mouse	2	16 ± 2·0
Portal vein†		mouse	2	9 ± 1·0
i.v.†		mouse	2	23 ± 3·5
Vena cava liver isolated†	(min)	rat		
Vena cava liver isolated	0	rat	4	10·5 ± 1·25
Vena cava liver isolated	3	rat	4	16 ± 1·0
Vena cava liver isolated	5	rat	4	21·5 ± 2·5
Vena cava liver isolated	7	rat	4	29 ± 4·0
Vena cava liver isolated	∞	rat	4	∞

* Times are minutes ± S.D. that salivation occurred after the administration of 5 mg/kg of slaframine. All tests include at least 4 animals per group.

† Animals were anesthetized with pentobarbital (100 mg/kg) and an abdominal incision was made for slaframine injections or sham operations.

TABLE 2. EFFECT OF VARIOUS INDUCERS AND INHIBITORS OF DRUG-METABOLIZING ENZYMES ON THE DELAY OF SALIVATION AFTER SLAFRAMINE ADMINISTRATION

Treatment	Dose (mg/kg)	Delay*
Control		15·3 ± 0·8
Slaframine†	5 (10 days)	16·8 ± 0·8
Phenobarbital†	100	8·8 ± 0·5
DDT†	30	8·0 ± 0·5
Chlordane†	40	8·0 ± 0·5
SKF-525-A‡	50	30 ± 1·0
SKF-8742-A‡	50	27 ± 2·0
DPEA‡	7·5	20 ± 1·0
Lilly 18947‡	50	27 ± 1·7

* Times are minutes ± S.D. that salivation started after the administration of slaframine (5 mg/kg). All tests included at least 4 mice per group and all injections were made i.p.

† The inducers were given daily for 5 days prior to testing with slaframine.

‡ The inhibitors were given 30 min prior to testing.

clearly shows that common inducers consistently reduced the delay in the induction of salivation, while inhibitors of drug-metabolizing enzymes consistently lengthened the delay. The inducers also resulted in a substantial decrease in the dose of slaframine required to elicit half maximal activity, while the inhibitors caused an increase in the dose required. It was interesting to note that the repeated administration of slaframine itself had no effect on the time required for its activation.

These results strongly suggest that slaframine requires activation by the liver before stimulating exocrine glands. It is also suggested that the enzymes involved in this activation are those responsible for the metabolism of most xenobiotics. Confirmation of these results will have to await isolation and characterization of the activated metabolite and activation *in vitro*.

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Department of Biochemistry
Michigan State University,
East Lansing, Mich. 48823, U.S.A.

STEVEN D. AUST

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Fluctuating levels of 5-hydroxytryptamine and histamine in neoplastic mast cells*

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RANDOM measurements showed that the levels of 5-hydroxytryptamine and histamine in neoplastic murine mast cells vary widely whether the cells are grown in culture or in the mouse.[1,2] These fluctuations were seen in cells derived from both the Dunn–Potter[1,2] and Furth tumors,[3] and were observed in descendants of the original tumor as well as in descendants of cloned, single cells. It seemed of interest to follow the levels of amines for a longer period of time to see if there was any obvious rhythmicity in these fluctuations.

Measurements of 5-hydroxytryptamine[4] and histamine[5] were made on descendants of the Dunn–Potter tumor, grown as ascitic tumors.[1–3] The levels of 5-hydroxytryptamine in these cells varied over the period of measurement (Fig. 1), ranging from 0·5 to 357 $\mu g/10^9$ cells in the X-2 line, from 0·1 to 608 $\mu g/10^9$ cells in X-1-C line, and from 0·2 to 2000 $\mu g/10^9$ cells in X-1-D. There was no clear rhythmicity in these fluctuations. Similarly, no rhythmicity was seen in the fluctuating histamine levels (Fig. 2), which varied in the X-2 line from 0·6 to 68·5 $\mu g/10^9$ cells, in X-1-C from 7·0 to 95 $\mu g/10^9$ cells, and in X-1-D from an undetectable level to 334 $\mu g/10^9$ cells. It is likely that the changing levels are due to fluctuations in the metabolism of the mast cells rather than of the mouse, since similar fluctuations were seen in cells grown in culture.[1]

The changing amine levels are probably reflective of changes in the capacities of the population of cells to take up and decarboxylate the amino acid precursors[1] of the amines, and perhaps also to the changing capacities to take up the preformed amines.[2,6] It is relevant that normal mast cells also showed[7] individual differences in their capacities to take up both the precursor amino acids and the amines.

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