# Insect Salivary Glands: Stimulation of Fluid Secretion by 5-Hydroxytryptamine and Adenosine-3′,5′-monophosphate

Abstract. *Low concentrations ($10^{-9}$M) of 5-hydroxytryptamine increase the rate of fluid secretion by isolated salivary glands of adult* Calliphora. *5-Hydroxytryptamine is present in* Calliphora *brain. Adenosine-3′,5′-monophosphate (cyclic AMP) also stimulates fluid secretion and may be involved in the mode of action of 5-hydroxytryptamine.*

The salivary glands of most insects secrete a watery saliva containing digestive enzymes (*1*). Although some information is available concerning the nature of enzyme synthesis and secretion, little is known about the formation and regulation of fluid secretion by these glands. We have studied fluid secretion of the salivary glands of the adult blowfly, *Calliphora erythrocephala*, using a technique developed for studying secretion in isolated Malpighian tubules (*2*).

The paired salivary glands are straight tubules which extend into the abdomen on either side of the gut. The two parts unite in the anterior region of the thorax to form a common salivary duct opening via the hypopharynx. The tubules from the abdomen were dissected out and placed in 20 $\mu$l of artificial medium (*3*) kept under liquid paraffin. A short length of tubule was pulled out of the medium by means of a fine silk thread ligatured around the cut end. Rate of saliva production was determined by periodically measuring the volume of saliva which escaped from an incision immediately behind the ligature. Tubules isolated in this way secrete saliva at a very slow rate (0.4 to $0.8 \times 10^{-3}$ mm$^3$/min). However, addition of 2 g of bovine albumin fraction V (Nutritional Biochemicals, control No. 8032) per 100 ml increased the rate of secretion 60-fold. Other proteins ($\gamma$-globulin, myoglobin, insulin, and horseradish peroxidase) have no effect. When bovine albumin is precipitated with 80 percent ethanol, the activity remains in the supernatant. Hence the stimulatory effect is due to a contaminant. The active factor can be dialyzed, is insoluble in organic solvents (ethanol, methanol, chloroform, and carbon tetrachloride), and is heat stable.

An active factor with properties similar to that found in bovine albumin has also been detected in the brains of adult *Calliphora* (and also in the brains of *Galleria* and diapausing Cecropia pupae). No activity was detected in fat body, which suggests that the factor may be confined to the nervous system. The active factor from any of these sources is not destroyed by digestion with pronase (3 hours incubation at 37°C, with pronase 1:100 by weight in 0.05*M* tris buffer, *p*H 7.8) or pepsin (3 hours incubation at 37°C, with pepsin 1:15 by weight in 0.01*N* HCl).

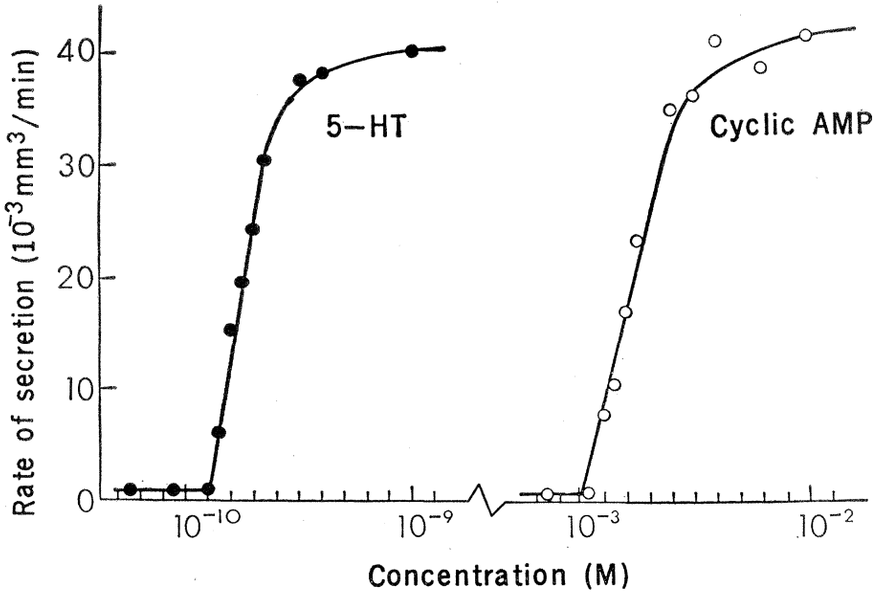


Fig. 1. Relationship between the concentration of 5-HT and cyclic AMP and the rate of fluid secretion by isolated salivary glands.

A clue to the identity of this active factor was provided by testing a variety of pharmacological agents over a concentration range of $10^{-3}$ to $10^{-6}$*M*. Salivary glands were insensitive to acetylcholine, pilocarpine, adrenaline, noradrenaline, histamine, and $\gamma$-aminobutyric acid, but were strongly stimulated by 5-hydroxytryptamine (5-HT). The possibility that the unknown factor might be 5-HT is strengthened by the fact that this substance normally occurs in the blood of vertebrates (the source of bovine albumin) and in insect brains (*4*).

The unknown factor from the extracts has properties similar to 5-HT when run on paper chromatograms. Bovine albumin and *Calliphora* brains were extracted with 80 percent ethanol and, after centrifugation, the supernatant was applied to chromatograms and run in either butanol, acetic acid, water (50 : 12 : 50) or pyridine, acetic acid, water (50 : 35 : 15). Since the amount of 5-HT in the unknowns was below the level of detection by the use of ultraviolet light (0.1 $\mu$g), its position on the chromatogram was determined by biological assay which could detect 0.0005 $\mu$g of 5-HT. Chromatograms were dried and each strip was divided into smaller parts, which were eluted in 2 ml of artificial medium and tested on isolated salivary glands. The active factor in both unknowns migrated as a single spot which had $R_F$ values very similar to that of 5-HT. Failure to visualize 5-HT in the unknown spots by ultraviolet light is due to the low concentration of this substance in bovine albumin and insect brain. Salivary glands can detect such low concentrations because they are exquisitely sensitive to this substance (Fig. 1). The steepness of the dose-response curve suggests that salivary glands could be used as a sensitive assay for 5-HT. Although further chemical confirmation of the active factor is required, the specific sensitivity of salivary glands to 5-HT strongly suggests that this agent (or a closely related tryptamine derivative) may function in the control of fluid secretion.

The control mechanism in insect salivary glands may thus resemble that found in various vertebrate epithelia which are also sensitive to biogenic amines; for example, acetylcholine stimulates vertebrate salivary glands (*5*) and histamine stimulates HCl secretion by the gastric mucosa (*6*). In the gastric mucosa, the stimulatory effect of histamine is mediated through

adenosine-3′,5′-monophosphate (cyclic AMP) which is a naturally occurring intracellular constituent (*7*). Indeed, cyclic AMP appears to mediate the effects of several hormones including the action of catecholamines and glucagon on liver, adrenocorticotropin on the adrenals, and vasopressin on toad bladder (*8*). In *Calliphora,* the effect of 5-HT on salivary glands may also be mediated by cyclic AMP because this substance can stimulate secretion (Fig. 1), whereas related compounds such as adenosine, adenylic acid, and adenosine triphosphate are ineffective over a similar range of concentration. The dose-response curve resembles that of 5-HT, except that the concentration of cyclic AMP needed is much greater. Where the intracellular levels of cyclic AMP have been measured, the concentration ranges between $10^{-8}$ and $10^{-6}M$ (*9*). Perhaps in salivary glands the cell membrane acts as a diffusion barrier to cyclic AMP and this accounts for the high concentration needed to stimulate secretion.

M. J. BERRIDGE
N. G. PATEL

*Department of Biology* and
*Developmental Biology Center,*
*Case Western Reserve University,*
*Cleveland, Ohio 44106*

### References and Notes

1. V. B. Wigglesworth, *The Principles of Insect Physiology* (Methuen, London, 1965), pp. 449–450.
2. J. A. Ramsay, *J. Exp. Biol.* **32**, 104 (1954); M. J. Berridge, *J. Insect Physiol.* **12**, 1523 (1966).
3. The artificial medium was prepared by dissolving the following substances in 100 ml of distilled water: NaCl (1.55 g), $NaH_2PO_4$ (32 mg), $Na_2HPO_4$ (54 mg), $CaCl_2$ (40 mg), $MgCl_26H_2O$ (400 mg), trehalose (360 mg), glucose (360 mg), glutamine (140 mg), α-alanine (80 mg), glycine (f00 mg), fumaric acid (100 mg), malic acid (100 mg), citric acid (100 mg), penicillin (6 mg), and streptomycin (20 mg). The *p*H of the medium was adjusted to 7.0 to 7.4 by titration with KOH. The final osmotic pressure of the medium was 350 milliosmoles.
4. S. Garattini and L. Valzelli, *Serotonin* (Elsevier, Amsterdam, 1965), pp. 56–58; E. H. Colhoun, in *Insects and Physiology*, J. W. L. Beament and J. E. Treherne, Eds. (Oliver and Boyd, London, 1967), p. 202.
5. H. W. Davenport, *Physiology of the Digestive Tract* (Year Book Medical Publishers, Chicago, 1966), p. 83.
6. D. Alonso and J. B. Harris, *Amer. J. Physiol.* **208**, 18 (1965).
7. J. B. Harris and D. Alonso, *Fed. Proc.* **24**, 1368 (1965).
8. F. Murad, Y.-M. Chi, T. W. Rall, E. W. Sutherland, *J. Biol. Chem.* **237**, 1233 (1962); E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.* **12**, 265 (1960); J. Orloff and J. S. Handler, *J. Clin. Invest.* **41**, 702 (1962).
9. R. W. Butcher and E. W. Sutherland, *J. Biol. Chem.* **237**, 1244 (1962).
10. Supported by NIH grants GM-09960 to Professor M. Locke, AM-09975-03 to Professor B. Schmidt-Nielsen, and H-6320 to Professor H. A. Schneiderman. We thank Professor M. Locke, Professor H. A. Schneiderman, and Dr. J. L. Oschman for reading the manuscript.

16 August 1968

## Intrastrain Differences in Serotonin and Norepinephrine in Discrete Areas of Rat Brain

Abstract. *Determination of levels of serotonin and norepinephrine in various brain areas of male Sprague-Dawley rats obtained from four different breeder-suppliers showed considerably different basal levels among the various groups, as well as differences in response to monoamine oxidase inhibitors.*

A considerable body of literature has evolved since the first reported measurements of serotonin (5-HT) (*1*) and norepinephrine (NE) (*2*) in mammalian brain. In general, most reports describe levels of the amines in whole brain or brain stem; very few reports have appeared on levels of 5-HT or NE in discrete brain areas. The picture is further complicated by reports confirming species and strain differences in brain amine levels (*3*), and by sensitivity limitations of methodology (*4*). We have examined the basal levels of 5-HT and NE in several discrete brain areas of Sprague-Dawley rats, using a more sensitive assay procedure developed in this laboratory (*4*). The results indicate considerable differences in levels of 5-HT and NE in brain areas of animals of the same strain obtained from different suppliers.

Adult, male Sprague-Dawley rats (60 to 80 days old, 300 to 350 g in body weight) were obtained from four commercial sources: Harlan Laboratories, Indianapolis, Hormone Assay Laboratories, Chicago, Simonsen Laboratories, St. Paul, and Windsor Biology Gardens, Bloomington. The animals were maintained with free access to Purina Lab Chow and water for at least 7 days prior to experimental use. After decapitation of the rats, brains were removed, areas dissected, and 5-HT and NE were determined as previously described (*4*).

The normal levels of 5-HT and NE in brain areas of rats obtained from the various sources are presented in Table 1. The differences are striking. Levels of 5-HT and NE are remarkably consistent in the cerebellum of the various groups. However, in other areas, considerable differences are seen in normal amine levels. Values for whole brain reflect largely the differences in the cerebral hemispheres, the largest portion of the whole brain weight. Further examination of rats obtained from two of these sources, Hormone Assay Laboratories and Simonsen Laboratories, indicate that significant ($P < .05$) differences exist for NE in cerebral hemispheres, hypothalamus-thalamus, and midbrain, and for 5-HT in midbrain and medulla.

Additional studies of the elevation in brain 5-HT and NE levels induced by administration of the monoamine oxidase inhibitor, pargyline (40 mg/kg, intraperitoneally), to Hormone Assay and Simonsen animals are also shown. Under these conditions, the effect on brain amines at 30 minutes after the

Table 1. Concentrations (μg/g) of serotonin and norepinephrine in areas of rat brain. Values are means ± standard deviations. Abbreviations: *N*, number; CH, cerebral hemisphere; HTH, hypothalamus; WB, whole brain.

| Supplier | *N* | Brain area | | | | | |
|---|---|---|---|---|---|---|---|
| | | CH | Cerebellum | HTH | Midbrain | Medulla | WB |
| | | *Serotonin* | | | | | |
| Harlan | 8 | 0.77 ± .04 | 0.24 ± .01 | 1.49 ± .44 | 1.05 ± .10 | 0.91 ± .07 | 0.76 ± .09 |
| Hormone Assay | 7 | 0.86 ± .05 | 0.24 ± .02 | 1.40 ± .10 | 1.35 ± .09 | 1.23 ± .10 | 0.88 ± .07 |
| Simonsen | 8 | 0.79 ± .07 | 0.22 ± .01 | 1.41 ± .10 | 1.16 ± .13 | 0.98 ± .13 | 0.79 ± .07 |
| Windsor | 4 | 0.89 ± .04 | 0.27 ± .02 | 1.48 ± .45 | 1.30 ± .05 | 1.23 ± .09 | 0.89 ± .05 |
| Hormone Assay + pargyline | 3 | 1.13 ± .09 | 0.29 ± .02 | 2.20 ± .10 | 1.77 ± .14 | 1.39 ± .13 | 1.14 ± .06 |
| Simonsen + pargyline | 3 | 1.08 ± .11 | 0.26 ± .04 | 1.91 ± .09 | 1.60 ± .09 | 1.26 ± .10 | 1.07 ± .09 |
| | | *Norepineprine* | | | | | |
| Harlan | 8 | 0.40 ± .03 | 0.19 ± .02 | 2.37 ± .51 | 0.67 ± .05 | 0.70 ± .13 | 0.48 ± .05 |
| Hormone Assay | 7 | 0.57 ± .04 | 0.22 ± .04 | 3.12 ± .44 | 0.95 ± .07 | 1.07 ± .10 | 0.67 ± .05 |
| Simonsen | 8 | 0.43 ± .07 | 0.20 ± .03 | 2.01 ± .28 | 0.80 ± .09 | 0.93 ± .17 | 0.54 ± .07 |
| Windsor | 4 | 0.51 ± .07 | 0.21 ± .02 | 2.90 ± .44 | 0.83 ± .06 | 0.94 ± .06 | 0.60 ± .07 |
| Hormone Assay + pargyline | 3 | 0.68 ± .01 | 0.29 ± .04 | 3.34 ± .61 | 1.13 ± .07 | 1.14 ± .04 | 0.79 ± .03 |
| Simonsen + pargyline | 3 | 0.49 ± .04 | 0.29 ± .16 | 2.15 ± .51 | 0.91 ± .13 | 0.92 ± .12 | 0.59 ± .08 |