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Concentration of Serotonin in Intestine and Factors Affecting Its Release[1] (38729)

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(Introduced by P. Griminger)

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It has been recognized for some time that mammalian and avian intestine are particularly rich in serotonin. By use of histochemical, histofluorescent, and radioautographic techniques this biogenic amine has been localized primarily in the enterochromaffin cells and to a lesser extent in the myenteric plexus (1, 8, 10). Serotonin in the blood is derived primarily from the intestine (6, 16). Page (13) has suggested that this flowing reservoir could provide neurohumors for the local control of circulation and aid in homeostasis. Certainly the factors that influence the release of intestinal serotonin into the vasculature should be physiologically important.

Toh (19) was the first to perfuse intestine through its vasculature, collect the perfusate, and measure it for serotonin. Bioassays did not reveal any serotonin in the perfusate. Bulbring and Crema (2, 3), observed a continual release of serotonin from the isolated intestine of guinea pigs into the lumen and found a direct relationship between the intraluminal pressure and the amount of serotonin released. They also found that 5-hydroxytryptamine (5HT) release was greater during periods of peristalsis than during intervening periods of rest, and suggested the possibility of 5-HT initiating the peristalsis. Hydrochloric acid was reported (14, 15) to release serotonin from the intestine both *in vitro* and *in vivo*. Burks and Long (4, 5) working with an isolated dog intestinal preparation observed a continuous release of serotonin into the vasculature. The amount of serotonin released was increased by administration of acetylcholine, epinephrine, norepinephrine, scratching the serosal surface, and increasing the intraluminal pressure.

The objective of these studies were (1) to establish the levels of serotonin in the small intestine of the domestic fowl, and (2) to determine what parameters may influence the release of serotonin from the intestine into the vasculature.

Materials and Methods. All birds used were mature White Leghorn chickens (*Gallus domesticus*). They were housed in individual cages in batteries and all were subject to the same controlled environmental conditions. Water was given ad lib. but the animals were denied food for 10 hr before being sacrificed. Pilot studies indicated that the short-term denial of food did not appreciably change the content of serotonin in the intestine.

Intestinal levels of serotonin. Eight males and eight females were sacrificed at the same time of day to avoid complications that might arise from possible diurnal rhythms of serotonin concentrations, which have been observed in other tissues (11). The body cavity was opened and the entire small intestine removed. Fat was cut away from the intestinal tissue which was then immersed in liquid nitrogen and stored at −29° until assayed. Whole intestines were assayed using a modification of the fluorometric chemical method of Weissbach *et al.* (20). Basically it involves homogenizing the tissue in 0.1 *N* HCl, centrifuging the homogenate and collecting the supernatant. An aliquot of the supernatant is then taken, proteins precipitated out, and 1 ml of this new supernatant is added to concentrated HCl and its fluorescence read in an Aminco-Bowman spectrophotofluorometer at an excitation wavelength of 290 nm and emission wavelength of 543 nm. Concentrations of intestinal serotonin

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obtained in this procedure agree with results obtained in our laboratory with the more lengthy fluorometric procedure of Wise (21, 22) which involves extraction of serotonin first into butanol and eventually into 0.1 *N* HCl.

Isolated intestinal preparation. Birds were given heparin (1000 units) intravenously and then sacrificed by cervical dislocation. The body cavity was quickly opened and the viscera exposed. The small intestine was cut just posterior to the duodenal loop and anterior to the caeca, and excised from the bird with the vasculature intact up to and including the anterior mesenteric artery and vein. The anterior mesenteric artery was cannulated and the intestine suspended at each end from a stainless-steel hook. Some of the posterior part of the intestine was cut and removed so that a 20- to 30-cm section remained. Vessels at each end of the intestinal section that had to be cut to free the tissue were ligated. The intestine was perfused with Hanks' solution which is a modification of Ringers solution. The perfusate was made with distilled-deionized water. It was bubbled continuously with 95% O_2–5% CO_2, and maintained at 40°. The perfusate was pumped with a Holter pump which maintains a constant flow and is not affected by back pressures up to 300 mmHg. The perfusate was collected at 30-sec intervals, immediately frozen, and stored at −29° until assayed.

Serotonin in the collected perfusate was fluorometrically assayed by the method previously described (20). All chemicals were introduced into the perfusate tubing in a 0.5-ml bolus and were made up to have approximately the same osmolarity as the Hanks' solution.

The following chemicals were tested for a releasing effect on intestinal serotonin: acetylcholine chloride, L-epinephrine bitartrate, L-arterenol bitartrate (L-norepinephrine bitartrate), glycine, and histamine dihydrochloride. All drug dosages refer to the base of the compound. Direct electrical stimulation of the intestinal tissue was utilized to determine if nonchemically-induced increases in intestinal motility affects the release of serotonin. As a corollary to this work, male and female intestines were compared both in amounts of serotonin released from the intestine under control conditions and in response to acetylcholine (Table II). The results are expressed as μg 5-HT/ml/min/g of tissue.

Other results are expressed as μg of 5-HT released into the perfusate during a 30-sec period, since only paired comparisons were involved where each intestine served as its own control (Table III).

Results. The concentrations of serotonin in the female intestine (10.79 ± 0.71 μg 5-HT/g wet tissue) were significantly greater than the male levels (7.98 ± 0.62) ($P < 0.02$) (Table I). The variability of the concentrations as indicated by the standard errors was approximately the same for both sexes.

The intestine continually releases serotonin into the vasculature at the rate of approximately 10 ng per minute per gram of tissue. Even after an hour of perfusion, release of serotonin of approximately the same magnitude was still observed, indicating that the preparation was fairly long lasting and

TABLE I. LEVELS OF SEROTONIN (5-HT) IN MATURE MALE (M) AND FEMALE (F) WHITE LEGHORN CHICKEN SMALL INTESTINES.

Sex	No. of birds	Serotonin content (μg 5-HT/g tissue; Mean and SEM)[a]
M	8	7.98 ± .62
F	8	10.79 ± .71

[a] Male and female values significantly different ($P < 0.02$).

TABLE II. EFFECT OF ACETYLCHOLINE (ACH) ON RELEASE OF SEROTONIN INTO THE ISOLATED INTESTINAL VASCULATURE OF MALES (M) AND FEMALES (F).

			5-HT released into the perfusate during 30-sec periods		
Dose (μg)	No. of birds	Sex	Control (before Ach)	0–30 sec (after Ach)	30–60 sec
2	10	M	2.42±.25[a]	6.86±.88	2.60±.08
2	10	F	3.07±.32	7.90±.61	3.76±.47

[a] μg 5-HT (ml × min × g tissue)$^{-1}$ (10^{-3}) ± SEM.

TABLE III. Effect of Various Chemicals on Release of Serotonin into the Isolated Intestinal Vasculature of Males.

Chemical	No. of birds	5-HT released into the perfusate during 30-sec periods (μg) Control (before agent)	0–30 sec (after agent)	30–60 sec
Glycine 11 mg	4	.095	.120	.105
		±.01	±.01[a]	±.01
Epinephrine 5 μg	10	.11	.128	.104
		±.007	±.009[a]	±.007
Norepinephrine 5 μg	11	.104	.128	.101
		±.011	±.012[a]	±.009
Histamine 5.5 μg	2	.08	.07	.07
Histamine 11 μg	6	.085	.085	.072
		±.014	±.014	±.015

[a] Control and 0- to 30-sec values are significantly different.

stable. However, most experiments were completed within the first 20 min of perfusion. Several of the chemicals significantly increased the amount of serotonin released, the greatest increase in release occurring upon administration of 2 μg acetylcholine (Table II) ($P < 0.001$). Several of the other chemicals also significantly increased the release of serotonin: 11 mg glycine ($P < 0.005$), 5 μg epinephrine ($P < 0.01$), and 5 μg norepinephrine ($P < 0.02$) (Table III). On the other hand, histamine (5.5 and 11 μg) (Table III) and electrical stimulation (at voltages ranging from 11 to 50 V) did not cause an increase in serotonin release although both caused a visible increase in intestinal motility. Interestingly, even though the concentrations of serotonin in the intestine were higher in females, there was no significant difference in the release of serotonin in males and females of the controls and those administered acetylcholine ($P < 0.05$ and $P < 0.50$, respectively).

Discussion. The small intestine represents the fifth tissue of the domestic fowl in which female levels of serotonin were shown to be significantly higher than in males. Spleen, blood (17), pineal (11), and brain (9) exhibited this same sex difference. Almost all of the serotonin content of the blood in the domestic fowl is found in the buffy coat fraction mainly in the thrombocytes and lymphocytes (12). The intestine is reputed to be the basic supplier of serotonin to the blood (7, 16). It appears that the sex difference in concentration of serotonin found in many tissues may depend on the sex difference in serotonin found in the intestine, with the female producing and releasing greater quantities of this chemical into the blood than the male. Another possibility is that a sex difference could exist in the binding and uptake properties of the blood elements that accumulate serotonin and this could account for higher levels of serotonin in the blood of females. It is interesting that there is no sex difference in the release of serotonin in the isolated preparation. The higher levels of serotonin in the intestine of females suggest that the female has the capability of releasing more serotonin. It is possible that agents or parameters that increase the release of serotonin from the intestine, such as the catecholamines, could be in greater concentration or supply in female blood and thus cause a continually greater release in the females. For example, Sturkie and Lin (18) have shown the female domestic fowl to have higher levels of norepinephrine in the plasma than the males. Agents and parameters not included in this study may also be involved.

Whether serotonin release has an immediate physiological function on the intestine or other organs is not clear. This work has shown that some chemicals and agents normally found in blood can affect the release of serotonin from the intestine. Whether this release of serotonin induced by blood-borne agents is an integral part of the normal physiology of the animal remains to be proved.

Summary. The concentration of serotonin in the female chicken intestine (10.79 μg/g wet tissue) was significantly higher than in the male (7.98 μg/g).

The release of serotonin from an isolated perfused intestine was studied. The intestine continually releases serotonin at the rate of 10 ng per g/min. A number of substances, including glycine, epinephrine, and acetylcholine, caused a significant release of

serotonin, with acetylcholine having the greatest effect. Histamine or electrical stimulation did not affect the release of serotonin.

1. Bendett, E. P., and Wong, R. L., J. Exp. Med. **105**, 509 (1957).
2. Bulbring, E., and Crema, A., J. Physiol. **146**, 18 (1959a).
3. Bulbring, E., and Crema, A., J. Physiol. **146**, 29 (1959b).
4. Burks, T. F., and Long, J. P., J. Pharm. Sci. **55**, 1383 (1966a).
5. Burks, T. F., and Long, J. P., Amer. J. Physiol. **211**, 619 (1966b).
6. Erspamer, V., J. Physiol. **133**, 1 (1956).
7. Erspamer, V., and Testini, A., J. Pharm. Pharmacol. **11**, 618 (1959).
8. Gershon, M. D., and Ross, L. L., J. Physiol. **186**, 477 (1966).
9. Gross, K. B., Ph.D. Thesis, Rutgers University, New Brunswick, NJ (1974).
10. Hammarstrom, L., Ritzen, M., and Ullberg, S., Experentia **22**, 213 (1966).
11. Meyer, D. C., Sturkie, P. D., and Gross, K., Comp. Biochem. Physiol. **46A**, 619 (1973).
12. Meyer, D. C. and Sturkie, P. D., Proc. Soc. Exp. Biol. Med. **147**, 382 (1974).
13. Page, I. H., Yearbook Med. Publ. Chicago, IL (1968).
14. Resnick, R. H., and Gray, S. J., Gastroenterology **42**, 48 (1962a).
15. Resnick, R. H., and Gray, S. J., J. Lab. Clin. Med. **59**, 462 (1962b).
16. Stacey, R. S., and Young, I. M., Biochem. Pharmacol. **13**, 129 (1964).
17. Sturkie, P. D., Woods, J. J., and Meyer, D., Proc. Soc. Exp. Biol. Med. **139**, 364 (1972).
18. Sturkie, P. D., and Lin, Y. C., Comp. Biochem. Physiol. **24**, 1073 (1968).
19. Toh, C. C., J. Physiol. **126**, 248 (1954).
20. Weissbach, H., Waalkes, T. P., and Udenfriend, S., J. Biol. Chem. **230**, 864 (1958).
21. Wise, C. D., Anal. Biochem. **18**, 94 (1967a).
22. Wise, C. D., Anal. Biochem. **20**, 369 (1967b).

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