Explor Anim Med Res,
Vol.4, Issue - 1, 2014, p. 8-18

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Review Article

SEROTONIN ODYSSEY IN INDIA

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Serotonin (5-hydroxytryptamine, 5-HT) was discovered 70 years ago and the study of serotonin and its receptors persist to acquiesce novel biological insights of medical importance in virtually all major organ systems, including the cardiovascular, pulmonary, gastrointestinal (GI), and genitourinary systems as well as the central nervous system (CNS) (Erspamer 1940, Xiao et al. 1998). Serotonin and serotonin receptors are important in the regulation of virtually all brain functions, and dysregulation of the serotonergic system has been implicated in the pathogenesis of many psychiatric and neurological disorders. A greater understanding of serotonin function has emerged during the last two decades with the cloning of at least 15 serotonin receptors, which are grouped into seven families based on signaling mechanisms. Other important advances have included the subsequent development of receptor-specific knockout mice, and the development of receptor subtype-selective drugs. These advances have also shown us that serotonin has critically important functions in many human organ systems outside the CNS, including the regulation of energy balance and food intake, GI and endocrine function, and cardiovascular and pulmonary physiology. These findings may help explain the diverse side effects of serotonergic drugs—from diabetes and metabolic syndrome to valvular heart disease. Interestingly the gastrointestinal tract (GIT) of our body resides 95% of the serotonin (5-HT) of the total body in the enterochromaffin cells (EC). This warrants for a great deal of research interest towards its significance in gastrointestinal pharmacology.

The Indian version of Serotonin odyssey was initiated in early sixties of the last decade to explore the natural product like banana (Fig.1) and other plants products to protect the various gastric complications induced due to the use of synthetic analgesics and even antacids like proton pump inhibitors. Later on detailed study was started the on banana in phenyl butazone induced ulcer and restraint ulcer in animal model experimentally (West 1958, Waalkes *et al.*, 1958, Sinha *et al.*, 1961, Sanyal *et al.*, 1964, 1965). Exploratory observational clinical study on radiologically diagonsed duodenal ulcer patients was carried out with banana powder to record preliminary clinical efficacy. Afterwards, detailed experimental and clinical study concerning banana has been undertaken, which is known to be a rich source of 5-HT naturally. The experiments were carried out and ultimately the vision on banana research shifted to serotonin research mission.

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Working hypothesis:

To designate a substance like 5-HT as physiological inhibitor of gastric secretion it must be present locally endowed with synthesizing and degradation enzyme system. A definite relationship of the inhibitory substance with the gastrin should also exist. Stimulatory and inhibitory forces interact and maintain balance of normal gastric secretion. Specific drugs affecting 5-HT metabolism and receptor activity must change the gastric secretary pattern either to hyper or hypo acid secretion. In clinical situation also acid secretion should correlate with hyper or hypo serotenemia. The drugs acting on 5-HT metabolism and receptor activity in discrete area like GIT may have alter the tissue status and function (Debnath 1977).

Clinical correlation:

To designate 5-HT as physiological gastric secretion inhibitor clinical correlation must be evident with its increased and/ or decreased level must reflect on the gastric secretary status hypo or hyper acid secretion. The proposition suggested that gastritis, duodenal ulcer, non-ulcer dyspepsia and some anorexic patients without obvious pathology either associated with diarrhea or vomiting could be grouped under Amlapitta. The cardinal symptoms are avipaka (dyspepsia). hrillash (nausea), udgara (eructation), amla (sour throat), shoola (epigastric pain), gurukosthavam (abdominal discomfort), antrakujana (gurgling), adhamana (tympanitis), shiroshoola (headache) match with the features of Amlapitta (gastritis syndrome). Besides those common symptoms vamana (vomiting) and dravamala (diarrhea) are the differentiating feature of urdhaga and adhoga amlapitta, may be correlated with hyper or hypo acid secretion found on evaluation of gastric function test along with 5-HT status in blood (Rakshit and Dutta 1975).

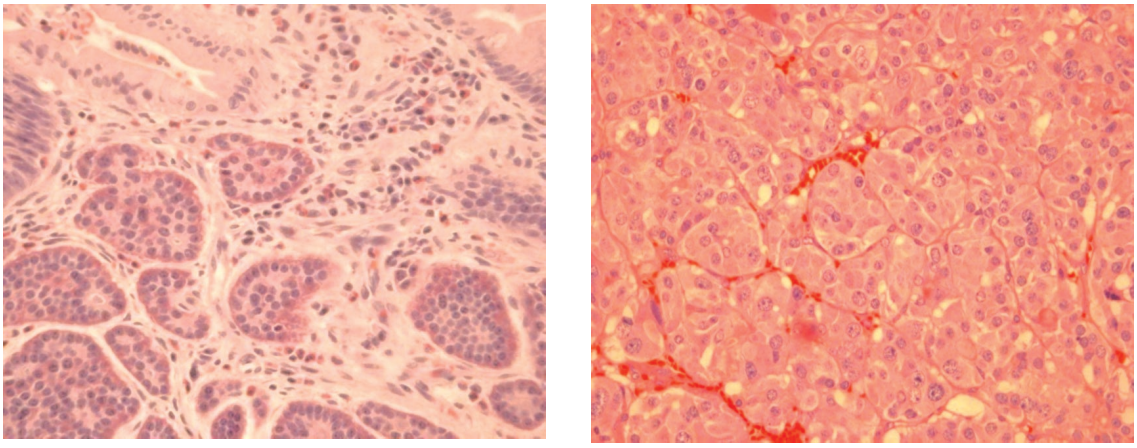
The clinical correlation study was designed partaking the group of patients diagnosed as amlapitta. The symptomatologies of patients in the study were carcinoid tumor and medullar thyroid carcinoma with features of diarrhea and hyper serotenemia independent of unknown gastric acid status. The diagnosis was confirmed histopathologically from biopsy (Fig. 2). Further, gastritis, anorexia, represent hypo acid secretion while with hyper acid secretion was correlative of duodenal ulcer and non-ulcer dyspepsia. Both the tumor and non tumor group of patients exhibiting with anorexia, heartburn, headache, fainting, giddiness vomiting or diarrhea were included in the study. In all the patients'1 h of basal gastric acid secretion and serotonin level in blood were estimated. Hyper serotonemic patients those represented with hypo acid secretion were treated with cyproheptadine (5 mg t.d.s) and those with hypo-serotonemic and hyper-acid secretion were treated with banana (5 g, t.d.s). Cyproheptadine administration increased the food intake, acid secretion but decreased the plasma serotonin level.

Gastric function test and plasma serotonin were evaluated in all the patients. Hyper acid secretion is always accompanied by hypo serotenemia. Administration of banana powder reversed the situation by decreasing acid and increasing blood serotoninin in duodenal ulcer and non-ulcer dyspepsia patients. Unripe banana showed anti-ulcerogenic activity. Banana in stomach, *in situ* the reduction of acid concentration may be related with its serotonin content and physico-chemical properties of

banana powder like mucilage (Sanyal *et al.*, 1964 & 1965, Best and Nasser 1984), increased enterochromafin cell population (in raw Banana powder) remaining in stomach in situ could neutralize acid and after 30 min of drug administration reduced total acid concentration significantly. Banana stimulates eicosanoid activity in human stomach tissue. There are reports that injudicious use of banana produce transient self limiting diarrhea and 5-HT2 antagonist ondensetron could check the diarrhea. In the present study it was observed that after treatment with banana powder enterochromaffin cell population in the stomach increased. Single and repeated ingestion of Banana elevated 5-HT in whole blood, indicating dietary influences on platelet 5-HT . The clinical features of both the group of patients correlated well with the known features of Amlapitta described in ancient times in Ayurveda, when gastric function test was not available (Vaidya 1982).

Fig.1: Banana (*Musa paradisiac)*

The study validates the choice for selecting Amlapitta as the clinical model to study the role of 5-HT on gastric secretion. The possible role of PGE1 and 5-HT in carcinoid tumor and medullar thyroid carcinoma to produce diarrhea became apparent by their increased number of



A B

Fig.2: Histopathology of Carcinoid and Medullar Thyroid Carcinoma.
(A) Showing increase population of enterochromaffin cells and medullar Thyroid carcinoma
(B) Para follicular C cells (in high power).

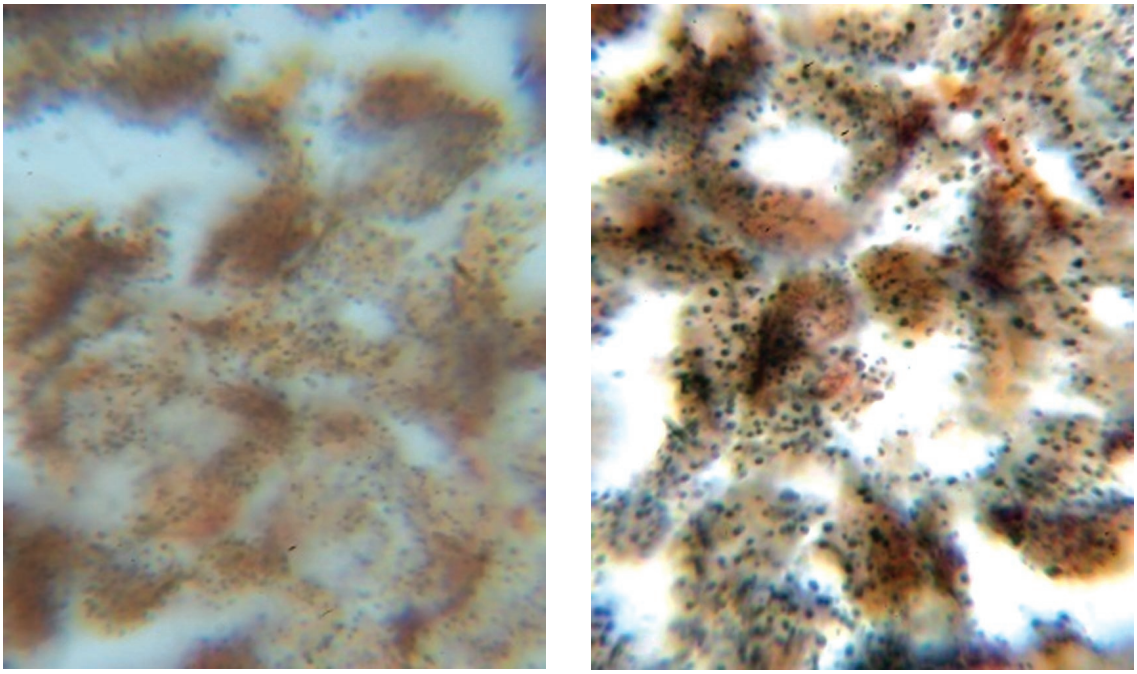


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Fig. 3: Enterochromaffin cell population of banana.
(A) Normal rat EC population **(B)** After treatment with Banana

enterochromaffin cells (EC) and parafilicular cells those secreting 5-HT and PGE1. Prostaglandins inhibits gastric secretion like 5-HT that too correlated well clinically. In further studies Prostaglandins (PGs) were also included to clear its role along with 5-HT in different experimental situation.

Banana treatment on enterochromaffin cell population in rats:

Enterochromaffin (EC) cell population in waster rats is shown in the Fig.3. After 7 days of banana treatment (5 g/kg oral) showed increased population of EC cell, suggested that banana treatment increased serotonin level by increasing the EC cell population.

Correlation of basal gastric secretion and serotonin:

Studies on gastric secretion and serotonin status in diseased conditions although, empowers 5-HT as gastric secretion inhibitor even then, validation of correlation on basal gastric secretion in normal physiological condition became essential to enrich the hypothesis in experimental animals and human studies with evidence.

Human study:

Gastric secretary pattern in neonates were studied at different time intervals(0- ½, ½ - 1, 2-4,4-8 h) after birth longitudinally and horizontally. The basal pH of gastric secretion

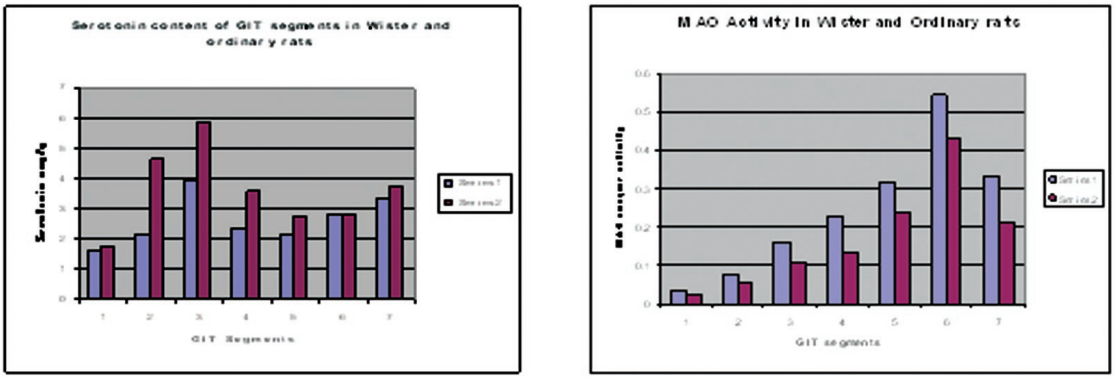

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Fig. 4: Serotonin and MAO activity in different strains of rat.

(A) Serotonin in different segments in GIT **(B)** MAO activity in different segments.

(Wister rats are grey colour, inbred rats are red color. 1,2,3 are Rumen, glandular and pyloric end of stomach, 4 isduodenum; 5 and 6 are proximal and distal ileum; 7 is ascending colon).

in full term, pre-term and small for babies are 2.0.4.0 and 6.0 respectively in gastric samples eight hours after birth (Debnath *et al.*, 1974 a,b,c, Ahmed *et al.*, 1977). Pentagastrin response were studied (5 ug/kg s.c) in full term neonates (2 days age) and on normal volunteers. The basal total gastric acid secretion remains low (in term of mEq /h) and serotonin level remained high in neonates in comparison to normal human adults. As the age progresses acid secretion increases and serotonin level decline. Pentagastrin response in full term neonates was poor than normal human volunteers.

Rat study:

For correlation, 5-Hydroxytryptamine, Mono-amine Oxidase (MAO) enzyme and gastric secretion were evaluated on two strain of rats. 5-HT and MAO enzyme were estimated on seven GIT segments of rats, stomach (rumen, gland and pyloric antrum,), duodenum (whole), ileum (proximal and distal), and colon (ascending). Among the seven segments highest concentration of 5-HT is found to be present in pyloric antrum and ascending colon, while MAO enzyme activity was lowest in pyloric antrum but highest in the glandular stomach and rumen. Reserpine, pCPA, decreased 5-HT level but 5-HTP and MAO inhibitor (pargyline and tranylcypromine) increased the 5-HT concentration in GIT as expected, while methysergide in lower dose (0.2 mg/kg) decreased and on higher dose (1.0 mg/kg) increased the 5-HT concentration in GIT (Debnath 1977). Results are Mean ± SE a, and b are statistically significant in comparison to Inbred control as $P<0.05$, <0.01 respectively. C –$P<0.05$ on 5-HT effect incomparison to Wister rats. In wistar strain of rats tissue 5-HT level is below the level of inbred rats.

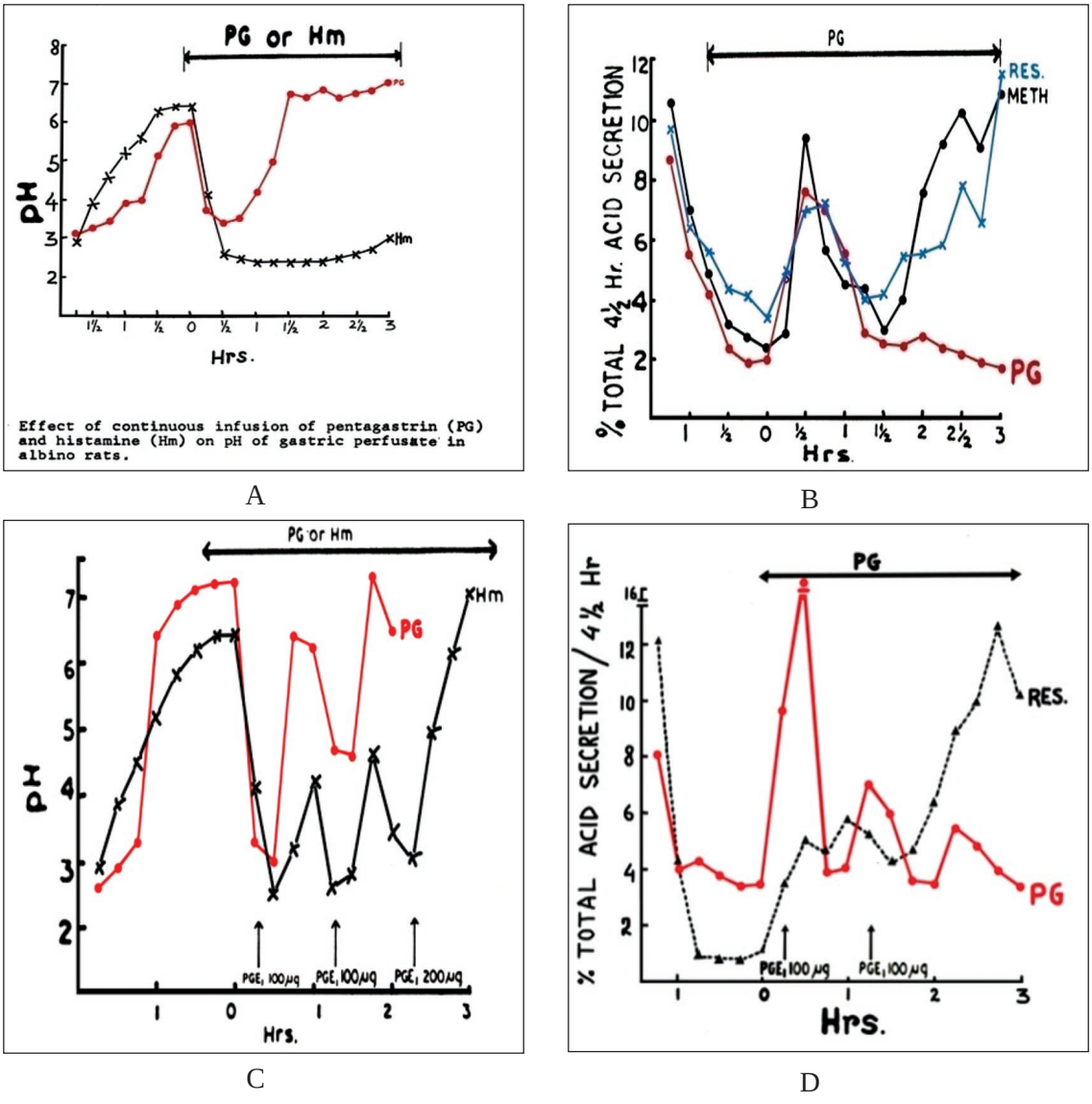

Fig. 5: Graph showing acid secretion status on Perfusion Experiments.

[(A) Pentagastrin and histamine continuous infusion and effect on pH fade phenomena, **(B)** Fade phenomena was not seen prior treatment with methysergide and reserpine, **(C)** Effect of prostaglandin E1 (100 µg/kg bolus) showed gastric secretion inhibition in both histamine and pentagastric stimulated gastric secretion, **(D)** In resepenized or pCPA treated rats continuous pentagastrin infusion in rats PGE1 failed to produce gastric secretion inhibition rather 2nd dose it reached to normal reserpinized level].

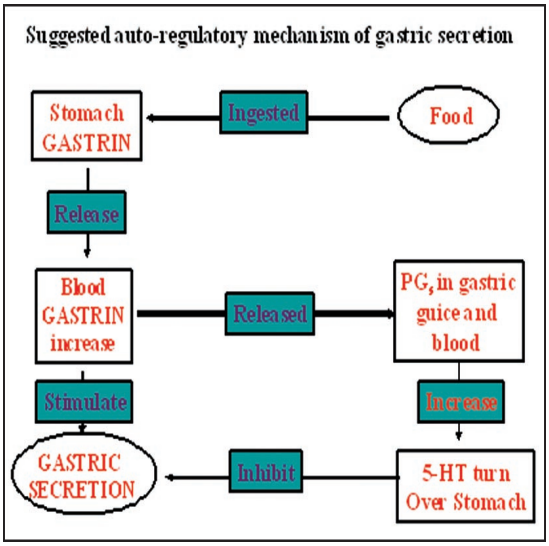

Fig.6: Hypothetical model of Serotonin mediated auto-regulatory mechanism in gastric secretion.

Conspicuously the basal gastric secretion is comparatively more than the inbred rats. The MAO activity of wister rats is comparatively more than inbred rats to keep 5-HT steady state level low. In this study direct correlation exists with the basal gastric secretion and 5-HT concentration status.

Experimental correlation:

In vindication of the hypothesis in the clinical model, and for confirmation in experimentally, Shay's rats, rat stomach perfusion and rat stomach fistula models' were utilized to observe the effects of different drugs and gastric secretagogue affecting 5-HT metabolism and receptor activity on gastric secretion.

5-HT and PGE1 on shay's rats gastric secretion:

5-HT and PGE1 dose dependently inhibited rat gastric acid and pepsin secretion Non-inhibitory dose of 5-HT (1 mg/kg) and PGE1 (0.1 mg/kg) when administered together potentiated the gastric inhibitory activity which could not be blocked with prior treatment with methysergide (0.1 mg/kg). Methysergide in lower doses (0.05, 0.1,0.2 mg/kg) stimulated gastric acid, pepsin secretion while in higher dose (1 and 2 mg/kg) inhibited acid secretion dose dependently. Drugs affecting 5-HT synthesis 5-HTP and MAO inhibitors (pargyline and tranylcypromine) inhibited gastric secretion. pCPA and reserpine although could decrease tissue 5-HT concentration in all segment but failed to stimulate gastric secretion, PGF2∝ in lower doses(0.1 and 0.4 mg/kg) stimulated secretion of acid pepsin while higher dose (1 mg/kg) inhibited gastric secretion . Stimulatory dose of PGF2∝ (0.04 mg/kg) failed to prevent inhibitory effect of PGE1 (0.4 mg/kg) and 5-HT (5 mg/kg), rather potentiating of inhibitory activity was observed (Guha *et al.*, 1979). Non-inhibitory dose of PGE1 on banana treated rats showed potentiation of inhibitory activity. Cyproheptadine treatment for 8 day showed stimulation of gastric acid secretion. The inhibitory effect of methysergide in higher doses could not be explained. This study validates clinical use of banana and cyproheptadine. Further, it suggests that the modulation of receptor activity is unable to prevent inhibitory activity of prostaglandins and 5-HT on gastric secretion.

Stomach perfusion experiment:

Perfusion Experiments was done on, fasted (24 h) wister rats (200-300 g) anaesthetized with urethane (1.6 g/kg im, controlled body temperature 35 ± 1 0 C). Drugs are injected through patent jugular vein and stomachs were perfused with N/2000 NaOH (pH 10.4) at

constant rate through slow injecting apparatus inserting tube through mouth. Perfusate are collected as 15 minute sample through polyethylene cannula at the pylorus and the rate was 10-10.5 ml/15 min. When consecutive two samples showed pH 3-3.5 again 4 samples at 15 min interval collected for 1 hour as basal rate of secretion. (Ghosh and Schild 1958).

In urethane anesthetized rats after continuous i.v administration of pentagastrin (5 µg/kg/h) peak acid secretion attained within 30-45 minutes which then started decline in spite of continued infusion of pentagastrin. The "fade" phenomena was completely blocked by previous treatment with reserpine and methysergide. This study confirmed the earlier report of in rats (Sanyal and Waton 1976). The results are summarized in Table 1 and 2 and Fig. 5. Further due to administration of prostaglandin E1 (100 µg/kg bolus) showed gastric secretion inhibition in both histamine and pentagastric stimulated gastric secretion (Fig. 5C). In resepenized or pCPA treated rats continuous pentagastrin infusion in rats PGE1 failed to produce gastric secretion inhibition rather 2nd dose it reached to normal reserpinized level.(Fig. 5D).Pattern of this rise after 2nd dose of PGE1 in acid output is more of less identical a depicted in reserpinized control rats. This suggests that without desired amount of 5-HT in the stomach PGE1 is unable to produce inhibitory activity. The results are shown in Fig.5 accorded to our hypothesis (Debnath *et al.,* 1978). Serotonin and MAO activity in different segments of digestive tract of wister and inbred rats are shown in Fig. 4.

Pentagastrin response on chronic fistula of rats:

(i) Pentagastrin response – Pentagastrin administration in conscious fistula rat's showed dose dependent stimulation of gastric secretion in volume, acid output and pepsin content. Pentagastrin (5 µg/kg.) administration by s.c route the peak stimulatory effect was observed within 30 min (15 min/2 samples) then started to decline. Pre-treatment with methysergide (0.1 mg/kg) and pCPA (300 mg/kg/3 days) followed by sc administration of pentagastrin the delayed decline (starts from 3rd or 4th sample) phase of gastric secretion observed. This study coroborated the earlier results an additional support to the hypothesis. Results are summarized in Fig. 5.

(ii) The effect of i.p administration of PGF2∝ in graded doses produced marked stimulation of gastric acid pepsin secretion. While, PGE1 in the graded doses (10 – 400 µg/kg) produced inhibition of gastric secretion. The effective minimum dose of PGF2∝ 50 µg/kg showed 50% increase, maximum stimulatory effect at 200 µg/kg, but PGF2∝ 400 µg/kg failed to stimulate any further.On the contrary PGE1 in higher doses 1 and 2 mg/kg produced inhibition of gastric secretion response. The stimulatory effect of PGF2∝ (50-400 µg/kg) could not be elicited by pretreatment with PGE1 (400 µg/kg), rather the inhibitory effect of the later was pronounced (Guha *et al.,* 1979).

Mechanism of action:

Goat liver feed (GLF) was prepared by the designated method. Small pieces of liver procured from market and washed three times with distilled water and then 5ml of 10% homogenate was administered p.o and thereafter 5-HT estimation in stomach, intestine and blood were carried out., att different time intervals. In experimental studies the choice of the dose and route of administration of PGE1,

Table 1: Pentagastrin response on Chronic fistula rat.

Parameters	n	Gastric secretion ml/h/200 g body weight		
		1st hour	2nd hour	3rd hour
Control	7	1.02 ± 0.32	0.98 ± 0.21	0.87 ± 0.27
Pentagastrin 5 µg/kg s.c	5	2.15 ± 0.41	1.68 ± 0.34	1.21 ± 0.25

Table 2: Effect of PGE1, Goat liver feed and Pentagastrin on 5-HT accumulation and decline rate in rat stomach and intestine.

Tissue	Group	Accumulation rate	Decline rate
Stomach	Control	3.24 ± 0.84	0.115 ± 0.06
	PGE_1	12.40 ± 1.34[b]	0.275 ±0.07
	GLF	5.70 ± 0.63[a]	0.180 ± 0.05
	Pentagastrin	7.16 ± 0.66[a]	0.281 ± 0.08
Intestine	Control	0.61 ± 0.08	0.107 ± 0.05
	PGE_1	0.41 ± 0.07	0.161 ± 0.07
	GLF	0.25 ± 0.05	0.114 ± 0.04
	Pentagastrin	0.42 ± 0.07	0.153 ± 0.06
Brain	Control	0.43 ± 0.07	0.382 ± 0.07
	PGE_1	0.84 ± 0.13[a]	0.327 ± 0.06

Results are Mean ± SE, a and b indicate statistical significant as a<0.05, b<0.01

(4.0 mg/kg s.c.) pargyline (75 mg/kg i.p.) pentagastrin (1 mg/kg s.c.) and pCPA (316 mg/kg i.p.), methysergide (1.0 mg/kg i.p 1 h) , reserpine (5 mg/kg i.p 16 h), were based on the earlier reports. Serotonin was estimated following the method of Snyder *et al.* (1965). Statistical significance was done by Student's unpaired't' test.

The rate of accumulation and decline of serotonin (after administration of pargyline-75 mg / kg .p. and pCPA 316 mg / kg, i p. respectively) were done according to the methods adopted by respectively (Tozer *et al.*, 1966, Neff and Tozer, 1968). To study the rate of accumulation, PGE1 (0.4 mg / kg s c.), GLF (5 ml) and pentagastrin (5 µg /kg) was administered along with pargyline and the animals were sacrificed after 30, 60 and 90 120 and 240 min where as, for studying the rate of decline PGE1 and GLF was administered 6 h after pCPA, and the animals were sacrificed at 6, 7, 8 ,9 and 10 h after pCPA treatment. The results are summarized in Table 15, 16, 17 and 18. Goat liver feed increased 5-HT in stomach nearly fourfold with in 30 min, and then started to decline. On the contrary, there was no

appreciable change in decline rate. Suggest that tissue 5-HT is available for decline of acid secretion attaining peak within 30-45 min after continuous stimulation with pentagastrin. The 5-HT synthesis rate increased in stomach and brain tissue with PGE1, GLF and pentagastrin but not in the intestine. Tissue steady state of 5-HT does not provide true picture on the tissue availability rate. In our experiments and reported studies. PGE1 did not increase steady state of tissue 5-HT, on the contrary increased synthesis rate. Goat liver feed increased fourfold steady state levels of tissue 5-HT while rate of 5-HT synthesis was less than pentagastrin and PGE1, indicate that natural food is slowly stimulating the acid secretion for long period to initiate digestion while pure secretagogue showed better reflection on the net tissue availability of 5-HT (Table 2). The hypothetical model of Serotonin mediated auto-regulatory mechanism gastric secretion is shown at Fig. 6.

CONCLUSION

After extensive research on the correlation of gastric secretion and serotonin status adopting different models in human and on experimental animals empowering different aspects, the knowledge vision on banana research shifted to serotonin research mission proved to be justified. The correlation studies in diseased condition, in neonates and normal adults, conscious and anaesthetized rats had put credence and the outcome, could validate the hypothesis. In vindication of the results of the experiments and clinical studies conducted so far in different laboratories further strengthens our proposition. In small bowel resection commonly hyper gastric secretion observed in man and in dog. It has been reported on duodenal acidification decrease in tissue 5-HT content which was pH dependent. In gastric ulcer and atrophic gastritis cases intake of breakfast increased prostaglandin levels, corresponding to abnormal neuro-humoral mechanism of gastric secretion.

ACKNOWLEDGEMENT

The author is thankful to Department of Science and Technology, Govt. of India, for providing fund for the project for Book Writing.

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***Cite this article as:** Debnath PK (2014) Serotonin odyssey in India. Explor Anim Med Res 4(1): 8-18.