Vestibulo-cerebellar participation in protection of duodenal mucosa: Possible role of neurotransmitters

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Vestibulo cerebellar lesion in rats produced a decrease in the intracellular presecreted mucus together with a decrease in the norepinephrine (NE) and serotonin (5HT) content of the duodenal tissue. Whereas vestibulo cerebellar stimulation by rotation produced an increase in the intracellular presecreted mucus and an increase in the NE content of the duodenal tissue but very little increase in 5HT content of the duodenum. The results suggest that the vestibulo cerebellum by modulating the tissue content of the neurotransmitter NE and 5HT has a direct influence in the protective mechanism through the intracellular mucus content.

Cerebellar influence over autonomic gastrointestinal expression has long been reported. Restricting areas of ablation demonstrated that the cerebellar mechanisms influencing autonomic GI function exist in nodulus and lower parts of uvula. Chemolesion of cerebellar nodule was shown to produce gastrointestinal pathology with ulceration. Intimate relationships between posterior cerebellum including the nodule with vestibular apparatus allow each other to influence in a reciprocal manner. Fibres of the vestibular system project to the vestibular nucleus, from there to the "vestibulo-cerebellum", cranial nerve nuclei and other centres. The vestibulo-cerebellum occupies a strategic position with anatomic links to limbic-hypothalamic-diencephalic and midbrain reticular medullar structures. Vestibular stimulation, a non-invasive and non-injurious technique, through the vestibulo-cerebellum corrects anhomeostatic conditions as well as various disorders. Sympathetic hyperactivity has been found to prevent gastric lesion formation. Acetylcholine aggravates acidity whereas intra-cerebroventricular injection of norepinephrine (NE) attenuates stress ulcer production. Serotonin (5-HT) is present in substantially high concentration in enterochromaffin (EC) cells of GI tract and thought to possess ulcerogenic properties. Mucosa of proximal duodenum has to encounter the aggression by acid and pepsin leaking from pyloric chamber. Moreover, biliary flux causes additional insults. While dealing with ulcerogenesis, most of the attention has been focussed on the acid component. Research pertaining to body's own protection against acid and pepsin has been much less in comparison. Most important aspect of this protection is the mucus secreted by epithelial cells, and much of which remains adhered as continuous sheath to the mucosal epithelial cell line of GI tract. In addition, bicarbonate secreted by the epithelia and retained by this mucus sheath plays great role in neutralizing the back-diffusing acid. Thus, the present study aims to explore relations of vestibulo-cerebellum, if any, with the indigenous 'mucus' protection against the aggressive factors.

Materials and Methods

Healthy adult Holtzman strain rats (96) of either sexes (150-200 g) were used and equally divided into: (a) control; (b) Sham control; (c) nodular lesion (NL) group; (d) vestibular stimulation (VS) group; (e) nodular lesioned animals receiving vestibular stimulation (LVS) and (f) Sham control animals receiving vestibular stimulation (SVS). They were caged individually and food pellet (Hindustan Lever Ltd.) and water was supplied ad libitum. The animals were sacrificed by cervical dislocation on 7th, 14th, 21st and 28th days from the date of beginning of experiment.

Making nodular lesion—Prior to surgery all the animals were fasted overnight but had free access to water. Rats were anaesthetized with sodium pentobarbital (40 mg/kg; ip, May Baker Co.). Each rat was placed in an Inco stereotaxic instrument equipped with ear bar that prevents damage to the tympanic membrane. Surgery was performed by a midline incision at the back of the head, the scalp was incisioned...
posteriorly in the midline and the adjacent pericranial muscles were retracted laterally. A burr hole of 1-2 mm was made on the posterior aspect of the scalp as per the stereotaxic coordinates of Pellegrino and Cushman \(^2\) \[AP = 12.8 \text{ mm}, (\text{d(depth)} = 6.8 \text{ mm}, L = 0.4 \text{ mm}\] so that the bipolar electrode could penetrate the required area of the cerebellum. Electrolytic lesions were made through this bipolar electrode, insulated, except for 0.4 mm at the tip. Direct current of 1-1.4 mAmp intensity and 20 sec duration was applied through Grass Lesion maker apparatus. After lesions were made the hole overlying the skull surface was closed with aseptic bone wax. After surgery all routine aseptic measures were taken and the animals were kept under observation. Particular care was taken for feeding till they recovered from surgical stress. All animals received 10,000 IU Penicillin post-operatively for three consecutive days by intramuscular route. NL was confirmed histologically.

**Vestibular stimulation technique**\(^2\) — Each rat was given VS of 15 min duration in two spells each day at 0900 hrs and 1900 hrs in a motor driven perspex drum which moved at a speed of 18 rpm at an angular acceleration of constant velocity rotation at 100\(^{\circ}\)/sec. The spinning of the drum was adjusted in such a way that both clockwise and anticlockwise movements consisting of successive 10 spins with angular acceleration achieved within 1 sec, followed by constant velocity and impulsive stop for 1 sec. VS by rotation of drum is considered to be a natural stimulation of vestibular system which can reflexly modulate the cerebellar function\(^1\).

**Sacrifice animal** — The animals were sacrificed on 7\(^{\text{th}}, 14\(^{\text{th}}, 21\(^{\text{st}}, \text{ and 28}\(^{\text{th}} \text{ days at 0800 hrs from the date of } \text{beginning of experiment with lethal dose of pentobarbital and duodenums were taken for biochemical estimation of NE and 5-HT content and for histological study by combined Alcian blue - PAS reaction for adhering epithelial mucus.}

Cerebells were processed for routine histology and 8-10\(\mu\)m thick sections were stained with haematoxylin and eosin for determination of lesion site.

**Biochemical estimation of 5-HT and NE**\(^2\) — Small pieces of proximal duodenal tissues were collected in cold condition, rinsed in cold saline and homogenised at 1000 g in 10 ml acidified butanol. Homogenate (4 ml) was mixed with 10 ml 10\% heptane and 5 ml 0.003 N HCl and then shaken for 5 min and centrifuged at 200 g for 10 min. Acid layer (4.5 ml) was eluted and mixed with 200 mg alumina and 1 ml of 2 M sodium-acetate. The mixture was shaken for 5 min and centrifuged at 200 g for 10 min.

Supernatant was taken for estimation of serotonin and precipitate was used for estimation of norepinephrine.

Supernatant was mixed with 3 volumes of 10\% isobutanol, shaken twice with equal volume of salt saturated buffer at pH 10. Then 2 volumes of 10% heptane added to the butanol phase and 5 ml of 0.1 N HCl was added and shaken well and then the mixture was made 0.3 N with respect to HCl. This was taken for estimation of 5-HT.

Cold distilled water (5 ml) was added to the precipitate and shaken well and then centrifuged at 2000 rpm for 30 sec. Then 3 ml of 0.33 N acetic acid was added and centrifuged at 2000 rpm for 3 min. Supernatant was transferred to glass stoppered centrifuge tube. Then 1.2 ml of freshly prepared ethylenediamine and ethylenediamine dihydrochloride mixture (7.5) was added to it and incubated at 50°C for 40 minutes. Mixture was cooled at room temperature and saturated with sodium chloride and then 4 ml 10% isobutanol was added. Then it was centrifuged at 2000 rpm for 3 min. The supernatant was thrown off and to the precipitate 4 ml of distilled water was added.

This was taken for estimation of NE, the fluorescence of 5-HT and NE was measured in the Perkin Elmer MPF 44B Fluorescence Spectrophotometer with activation wavelength set at 295 and 320 nm respectively and emission wavelength set at 550 and 450 nm respectively.

**Analysis of data** — All biochemical data were statistically analysed and the significance of differences between the mean values were assessed using two tailed \(t\)-test for paired samples.

**Results and Discussion**

**Motor behaviour following cerebellar lesion** — Movement disorders were noted in all the rats after lesions were made in the nodular cerebellum. It was observed that nodular lesions caused impairment of postural symmetry, abduction and hypertension of the hind limbs, swaying of the body movements, etching behaviour, crouching and loss of balance on beam walking tests. But such movement disorders persisted up to 4-5 days after lesion of cerebellum. Then gradual improvement was noted on motor movement and it returned to normal condition within 7 days after lesion.
Motor behaviour following VS—During VS, the motor reactions and responses of the individual rat when rotated to its right or left was easily visualized for the objective assessment of the animal's adaptability. Most animals stood up by hind limbs with neck extended and a few days of training rapidly adapted the rats to the rotating cage.

Histochemical study—Microphotographs (Figs 1-4) show reduction in histochemical reaction in intracellular presecreted mucus in the mucosal epithelial lining of proximal duodenum of NL animals in comparison to control. The adherent mucus disappeared during routine histological procedures. Whereas increased histochemical reaction for intracellular presecreted mucus in the mucosal epithelial lining was observed after vestibular stimulation.

Neurotransmitter (NE and 5HT)—Biochemical data (Table 1) show reduction in NE content of duodenal tissue in NL animals and increase in the VS group; the changes were significant on 21st day compared to control and steadied thereafter. The duodenal tissue content of NE increased in LVS when compared to the NL but was lower than the VS and the control group. The tissue content of 5-HT also decreased in NL animals significantly on 21st day, and remained same afterwards. In VS group 5-HT level did not change. The tissue content of 5-HT in LVS gradually increased from 7th day onward and reached its maximum on the 28th day. The 5-HT content of LVS animal on the 28th day was nearly same as the 5-HT content of the VS group on the 28th day.

Figs 1-4—(1) Mucosa of proximal duodenum from control animal. Luminal (mucosal) cell-lines of the villi are PAS positive as well as the muscularis mucosae underneath. (x 100); (2)—Mucosa of proximal duodenum from the NL group (21 days) shows reduction in PAS reaction at the surface mucosal cell-line, while muscularis layer retained stain. (x100); (3)—Mucosa from the VS group (21 days) shows abundant stain precipitation at the surface mucosal cell-line. (x100) and (4)—Proximal duodenum from LVS animal (28 days) [photomicrographs are Alcian blue-PAS x100].
Table 1—Changes in NE and 5-HT content (µg/100 mg) of duodenum
[Values are mean ± SE from 16 animals in each group]

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<thead>
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<th>Days</th>
<th>Control</th>
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<th>NL</th>
<th>VS</th>
<th>SVS</th>
<th>LVS</th>
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P values: *, # < 0.05
significant from control
# significant from NL counterparts.

Andrejik et al. have shown evidence of sympatheoexcitation after stimulation of the fastigial nucleus by vestibular stimulation.

The vestibular cerebellar nodule and adjacent parts of uvula have intimate connections with the vestibular apparatus and also with important brainstem nuclei and centres.

Vestibular stimulation increases the overall sympathetic tone increasing tissue content of NE while nodular lesion decreases tissue NE levels.

Pack et al. found sympathetic endings in human bronchial wall close enough to the mucous glands to influence their secretions, but they failed to record increased secretions after field stimulation. They found that NE applied in higher concentrations stimulated mucous secretion although lower doses (1μM) failed to produce any effect. Salivary secretion was found to be characteristically sparse with adrenergic stimulation. Observations by Guha and Maiti which suggested that sympatheoexcitation by vestibular stimulation increases tissue NE levels and intracellular pre-secreted mucous substantiating decreased secretion or output support the present observation. The reverse effects were found after nodular lesion, where tissue NE levels decreased along with the decrease in intracellular mucous content proving increased secretion.

However literature studies have shown that the mode of controlling the mucus output is probably different by 5-HT. Serotonin, produces differential effects; it decreases gastric mucosal mucus content in a dose dependent way and presumably possess ulcerogenic properties. In respiratory tract 5-HT has been found to induce mucus secretion and 5-HT was also found in secretions induced by histamine. The store house of 5-HT are the enterochromaffin cells (EC cells) which are distributed throughout the stomach and duodenum. Adrenergic endings have been reported in close proximity to EC cells and clonidine, a α2-agonist inhibited the release of 5-HT from EC cells. Therefore, it seems likely from the present study that increased NE levels due to VS inhibits the EC cells and inhibits the release of 5-HT from EC cell, thus restricting mucus turnover, maintaining its viscous, semi-solid, adherent nature which protects the duodenal mucosa. On the other hand, NL lowered NE levels resulting in the decreased inhibitory action of NE on EC cells which should concurrently increase 5-HT content. However NL also decreases EC cells and hence the total 5-HT content of duodenal tissues is lowered.

References
11 Wilson VJ, The labyrinth, the brain and posture, Am Sci, 63 (1975) 325.