

*Full Length Research Paper*

# Effect of acute bilateral adrenalectomy and reserpine on gastric mucus secretion and mucosal injury in pyloric ligated rats

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Accepted 24 November, 2019

Adrenalectomy produces many changes in gastric functional parameters including gastric acid secretion, gastric motility and mucosal blood flow. The present study was undertaken to assess the effect of acute bilateral adrenalectomy and reserpine on gastric mucus secretion and degradation in the pyloric ligated rats. Groups of Wistar rats of mixed sexes were adrenalectomized or administered with 5 mg/kg reserpine intraperitoneally or given both treatment followed by pyloric ligation. Adherent mucus was assessed by the alcian blue binding technique while erosion of the surface gel was assessed from the appearance of sialic acids and galactose in the gastric juice. Gastric mucosa injury was quantified by measuring the area of gastric mucosa damage. The amount of alcian blue bound to adherent mucus and soluble mucin concentration were not significantly affected by adrenalectomy ( $P > 0.05$ ). Reserpine diminished adherent mucus secretion and increased soluble glycoprotein in the gastric juice. Adrenalectomized animals (40%,  $n = 5$ ) developed haemorrhagic lesions as compared to 100% ( $n = 5$ ) in animals treated with reserpine. Adrenalectomy or propranolol did not protect the animals against the reserpine induced injury. It is concluded that the adrenal glands seem to be of no importance in the secretion of gastric mucus but reserpine inhibits mucus release and stimulates surface mucus gel breakdown via  $\alpha$ -adrenoceptor stimulation.

**Key words:** Adrenalectomy, gastric mucus, reserpine, sialic acid.

## INTRODUCTION

The entire gastrointestinal epithelium is covered by an adherent layer of visco-elastic mucus gel. The mucus is believed to be essential for the protection of gastric mucosa against injury by aggressive factors active in any stress situation (Allen and Flemström, 2005). The adherent mucus barrier is constantly being turned over as part of the protective functions of the mucosa surface. Thus, degradation of mucus is a normal feature of equilibrium between mucus synthesis, secretion of preformed mucus and breakdown of existing adherent mucus (Keogh et al., 1997). This dynamic balance must be regulated to ensure

a continual mucosa protection against potential damaging compounds. Various studies have indicated that various mechanisms are involved in the regulation of mucus metabolism in the gastric mucosa (Tabuchi et al., 1997; Takahashi and Okabe, 1998; Hotta, 2000). However, there is controversy about the role of adrenal glands in the regulation of gastric mucus secretion.

The absence of adrenal glands results in altered functions in many parts of the body including the gastrointestinal tract. It has been reported that adrenalectomy induces glucocorticoid deficiency in animals and such deficiency has been implicated in the development of mucosal injury (Filaretova et al., 2001a). There has been a suggestion that endogenous release of glucocorticoids during stress help gastric mucosa to resist harmful actions of both weak and strong ulcerogenic stimuli

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(Filaretova et al., 2001b). On the other hand there are also reports that associate the use of corticosteroids or adrenocorticotropin hormone (ACTH) to the formation of gastric ulcers (Roberts et al., 1978; Messer et al., 1983).

Adrenal derived catecholamines or dopamine may contribute to the pathogenesis of gastric or duodenal ulcers (Glavin and Szabo, 1990). Reserpine is a dopamine granule depleting agent in many organs including the brain and adrenal medulla (Sikiric et al., 2000). An *in vivo* infusion of dopamine reduced pentagastrin stimulated gastric acid secretion in man (Valuenzela et al., 1979) and rat (Parmar et al., 1984) and dopamine agonists were found to prevent the ulceration induced by pyloric ligation or stress (Sikiric et al., 1985). Although, the mechanisms underlying such actions remain unclear but the results of Salim (1991) suggests that reserpine cause vagal alpha-adrenoceptor stimulation to produce mucosal vasoconstriction and injury. Although, adrenal derived glucocorticoids and catecholamines have been suggested to prevent gastric mucosal injury, no study, however, has addressed the role of adrenal glands or reserpine on the secretion of gastric mucus in a system where glycoprotein erosion is measured together with adherent mucus secretion in the gastric mucosa in the unstimulated state. Therefore, the present study was undertaken to examine the effect of acute bilateral adrenalectomy and dopamine depletory agent (reserpine) on gastric mucus secretion and erosion in relation to gastric mucosal injury in the pyloric ligated rat.

## MATERIALS AND METHODS

### Animals

Wistar rats of mixed sexes weighing between 180 - 200 g were purchased from disease free animal house of Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were housed in separate large cages in group of 5 rats per cage in environmentally controlled room ( $25 \pm 2^\circ\text{C}$ , 12 h light/dark cycles) with free access to standard laboratory chow. Tap water was supplied *ad libitum*. The animals were deprived of feed but not water 18 h before the experiment. Animal studies were reviewed and approved by an Institutional Committee in accordance with National guidelines before the study.

### Drugs and chemicals

Sodium periodate, 2,4-dinitrophenylhydrazine (2, 4-DNPH) and sodium pyruvate were purchased from BDH Chemicals Ltd, Poole England. N-Acetylneuraminic acid, pig gastric mucin, periodic acid, thiobarbituric acid, urethane and other chemicals were purchased from Sigma Co. (St Louis, MO USA). Reserpine and propranolol were gifts from the Department of Pharmacology, Ahmadu Bello University, Zaria. All the drugs were diluted in normal saline and injected intraperitoneally (i.p.) in a volume of 1 ml per 100 g of body weight and were prepared fresh.

### Experimental design

Rats were divided into 6 groups and each group consisted of five rats.

**Group I:** It represented the sham control group. Animals in this group had their adrenals exposed but not removed.

**Group II:** Acute bilateral adrenalectomy was performed in these animals before pyloric ligation.

**Group III:** It represented the control group of animals administered with saline. This group differs from group I in that animals in this group were sacrificed 6 h after pyloric ligation while animals in group I were sacrificed 3 h after pyloric ligation.

**Group IV:** Reserpine (5 mg/kg i.p.) was administered to adrenal intact animals in this group.

**Group V:** Propranolol (1 mg/kg i.p.) was administered 30 min before reserpine (5 mg/kg i.p.) administration to adrenal intact animals in this group.

**Group VI:** Bilateral adrenalectomy was performed on animals in this group followed by administration of reserpine (5 mg/kg i.p.).

### Adrenalectomy

Acute bilateral adrenalectomy was performed according to the technique described by Gidener et al. (1996). After a midline incision under urethane anaesthesia (1.5 g/kg), the abdomen was opened and the adrenals were isolated and carefully removed with forceps (Group II). In the sham controls the abdomen was opened, the adrenals were exposed but not excised, and the incision was closed (Group I). Animals in both groups were sacrificed 3 h after pyloric ligation by cervical dislocation.

### Reserpine administration

Intraperitoneal (i.p.) injection of reserpine (5 mg/kg) was administered by slow infusion to adrenal intact (Group IV) or adrenalectomized (Group VI). In a final series of experiments, the effect of a potent  $\alpha$ -adrenergic receptor blocker, propranolol (1 mg/kg) to inhibit sympathetic nerves on reserpine reaction was determined. The drug was administered i.p. 30 min before reserpine [5 mg/kg] administration (Group V). Control animals received the vehicle (normal saline) only (Group III). In all the groups, pyloric ligation was performed immediately after reserpine or saline administration. The animals were sacrificed by cervical dislocation 6 h after pyloric ligation.

### Gastric mucosal damage

In all the experiments described above, the stomachs were removed, the gastric contents were carefully collected, measured and stored at  $-4^\circ\text{C}$  until analysed. Thereafter, the stomachs were opened along the lesser curvature, rinsed in cold saline, laid out on a flat surface and examined for ulcer score. Each individual haemorrhagic lesion was measured along its greatest length ( $< 1$  mm: rating of 1; 1 - 2 mm: rating of 2;  $> 2$  mm: rating according to their length in mm). The overall total was designated as lesion index (Holzer and Lippe, 1998). After the determination of ulcer score, the stomach was then processed for adherent mucus measurement.

### Measurement of adherent mucus secretion

Adherent gastric mucus was determined as described by Corne et al. (1994). The stomach was removed, opened along the lesser curvature and rinsed in cold saline. The glandular part of the stomach was excised, weighed and immersed for 2 h in 10 ml of 0.1% w/v Alcian blue in 0.16 M sucrose solution buffered with sodium acetate (pH 5.8). The excess dye was removed by two rinses in 0.25 M of sucrose (15 min each). The mucus-bound dye

**Table 1.** Effect of acute bilateral adrenalectomy (n = 5) and sham-operation (n = 5) on free sialic acid (FS), bound sialic acid (BS), galactose (GAL), total protein (PROT), adherent mucus (MUC) pyruvate sialate lyase activity (PSL) in pylorus ligated rats.

	FS (mg/ml)	BS (mg/ml)	GAL(mg/ml)	PROT (mg/ml)	MUC ( g/g of tissue)	PSL(mmoles/min)
Sham control	0.38 ± 0.09	1.15 ± 0.13	0.09 ± 0.01	0.2 ± 0.02	1.72 ± 0.11	0.10 ± 0.01
Adrenalectomy	0.63 ± 0.20*	1.11 ± 0.30	0.148 ± 0.02	0.33 ± 0.09	1.65 ± 0.12	1.89 ± 0.40**

The total activity of PSL is expressed as micromoles of pyruvate released per minute in the gastric juice. One unit of pyruvate sialate lyase activity is defined as the amount that releases 1 mmole of pyruvate per minute under the reaction conditions. Data shown represent mean ± SEM. \*P < 0.05, \*\*P < 0.001 compared with sham-operated control.

was extracted by immersing the gastric tissue in 0.5 M MgCl<sub>2</sub> solution, which was intermittently shaken for 1 min at 30 min intervals during a 2 h period.

The blue extract was shaken with diethyl ether. The emulsion was then centrifuged at 5000 g for 10 min and the optical density of the aqueous phase was measured spectrophotometrically at 605 nm. The amount of mucus adherent to the gastric mucosal was estimated from a standard curve established using concentrations from 0 to 20 g pig gastric mucin. The results were expressed as g mucin per gram of wet tissue (g/g of tissue).

#### Analysis of gastric samples

Samples of gastric secretions collected from all the animals in the series of experiments described above were assayed for total protein and free sialic acid (SIA). Total protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a protein standard, while free SIA was determined by the thiobarbituric acid assay method of Aminoff (1961).

As this assay measures only free SIA, gastric samples were heated at 80°C for 60 min in 0.1 N HCl to release bound SIA without degradation (Warren, 1959a, b). From the difference between the total and free SIA the bound SIA, corresponding to glycoprotein bound SIA was calculated.

Pyruvate sialate lyase (PSL) activity was assayed by colorimetric method for measuring the amount of pyruvate liberated by hydrolysis of SIA in the gastric juice using 2,4- dinitrophenylhydrazine (2,4- DNPH). Gastric juice samples were incubated with 0.00125% of 2,4-DNPH reagent for 10 min at 37°C (Ketter and Randle, 1998).

In each experiment, pyruvate at known concentrations was determined in parallel as standards.

Galactose was determined by the method of Duboise et al. (1956). The total amount of SIA, pyruvate and sugars present in each sample were estimated from standard curves prepared from appropriate standards.

#### Statistical analysis

Data were expressed as mean ± standard error of the mean (S.E.M.) and analyzed by using the SPSS statistical software package (version 10). Differences between groups were considered significant at P < 0.05. Differences between adrenalectomy and sham control groups were assessed with student t-test while the effects of reserpine on gastric secretions and mucosal injury were assessed with two-way analysis of variance (ANOVA).

Treatment (reserpine and saline injection) was considered a between-group factor, whereas the other treatments (adrenalectomy and reserpine, propranolol and reserpine) were considered as within-group factor.

## RESULTS

### Effect of bilateral adrenalectomy

As shown in Table 1, the effect of acute bilateral adrenalectomy on bound sialic acid (BS), galactose (GAL) and total protein (PROT) in gastric juice of pylorus ligated rats were not significantly different (P > 0.05) from control animals which had their adrenals intact except for FS (P < 0.05) and PSL total activity (P < 0.001) which were significantly higher in the adrenalectomized animals. Adrenalectomy did not modify the amount of adherent mucus (MUC) on the mucosa surface (Table 1).

Adrenalectomy led to haemorrhagic lesions in 40% of the animals with a mean ulcer index of 0.8 ± 0.01 while the sham control animals had no mucosal lesions.

### Effect of reserpine

The effect of administration of reserpine (5 mg/kg i.p.) on levels of gastric SIA, GAL, PROT and MUC 6 hours following treatment is shown in Table 1. Analysis of variance showed that reserpine significantly increased FS (F (3, 16) = 3.23, P = 0.02) and decreased BS (F (3, 16) = 3.23, P = 0.002). The decrease in GAL was not statistically significant (P > 0.05) when compared to control animals. The amount of PROT in the gastric juice was not affected by reserpine when compared to that of control animals (P = 0.05). All the sialic acids released by adrenalectomized animals administered with reserpine were in the free form (Table 1).

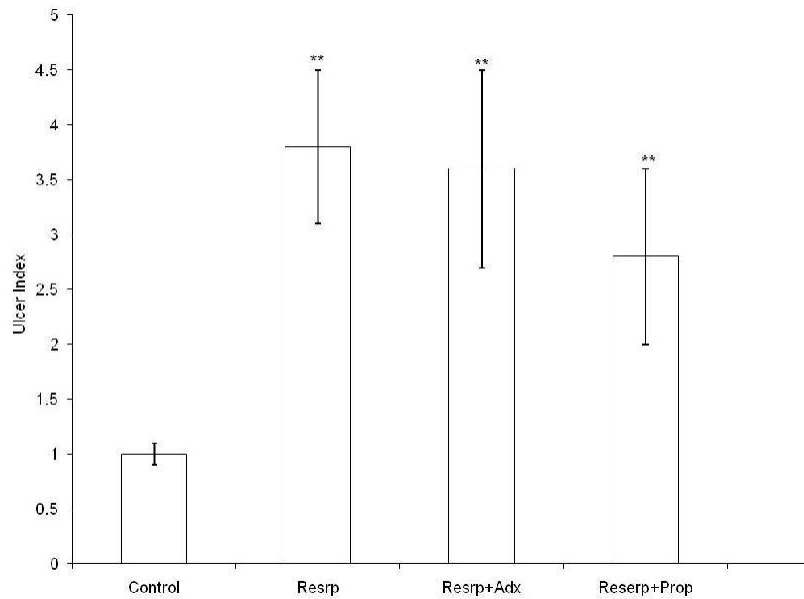
Reserpine induced increase in FS was inhibited by propranolol pre-treatment (1 g/kg) but the concentration of GAL was not affected by prior administration of propranolol. Total PROT output in adrenalectomized animals administered with reserpine was significantly reduced when compared to control animals (P < 0.05). The amount of adherent mucus was significantly decreased by i.p. injection of reserpine when compared to the controls (P < 0.001). This effect of reserpine was not modified by adrenalectomy but blocked by propranolol pre-treatment.

Intraperitoneal injection of reserpine significantly decreased PSL activity as indicated by decreased pyruvate

**Table 2.** Effects of reserpine, reserpine following adrenalectomy (reserpine + adx) and reserpine following propranolol pretreatment (reserpine + prop) on free sialic acid (FS), bound sialic acid (BS), galactose (GAL), total protein (PROT), adherent mucus (MUC) pyruvate sialate lyase activity (PSL) in pylorus ligated rats.

Groups	FS (mg/ml)	BS (mg/ml)	GAL (mg/ml)	PROT(mg/ml)	MUC ( g/g of tissue)	PSL(mmoles/min)
Control	0.78 ± 0.04 <sup>**</sup>	3.59 ± 0.06 <sup>**</sup>	0.13 ± 0.06	0.40 ± 0.08	1.99 ± 0.13	2.13 ± 0.17
Reserpine	2.06 ± 0.14 <sup>**</sup>	0.74 ± 0.04 <sup>**</sup>	0.09 ± 0.01	0.43 ± 0.05 <sup>*</sup>	0.82 ± 0.18 <sup>*</sup>	0.58 ± 0.12 <sup>**</sup>
Reserpine + Adx	2.01 ± 0.04 <sup>*</sup>	0 ± 0.0 <sup>**</sup>	0.07 ± 0.02	0.12 ± 0.05 <sup>*</sup>	1.03 ± 0.16	0.19 ± 0.09 <sup>**</sup>
Reserpine + Prop	0.31 ± 0.03 <sup>*</sup>	1.85 ± 0.05 <sup>*</sup>	0.04 ± 0.02	0.37 ± 0.05	2.06 ± 0.11	1.78 ± 0.14 <sup>*</sup>

The total activity of PSL is expressed as micromoles of pyruvate released per minute in the gastric juice. One unit of pyruvate sialate lyase activity is defined as the amount that releases 1 mmole of pyruvate per minute under the reaction conditions. Data shown represent mean ± SEM. \*P < 0.05, \*\*P < 0.001 compared with vehicle treated group (control).



**Figure 1.** Effect of 5 mg/kg reserpine (Resrp), adrenalectomy followed by reserpine (Resrp + Adx) and reserpine following propranolol (1 mg/kg) pretreatment (Reserp + Prop) on gastric mucosal lesions in pylorus ligated rats. Data shown represent mean ± SEM. \*\*P < 0.001 compared with controls groups.

concentration in the gastric juice (Table 2). The effect of reserpine on PSL was enhanced by adrenalectomy and diminished by prior administration of propranolol.

Reserpine treated animals developed oval or rounded lesions confined to the glandular stomach. The mean ulcer index is shown in Figure 1. The effect of reserpine on the ulcer index and ulcer incidence was not significantly affected by adrenalectomy or propranolol pretreatment (P > 0.05).

## DISCUSSION

The results obtained from this study have shown that the adherent mucus on the mucosa surface was not affected

by adrenalectomy but there were measurable amounts of soluble mucus glycoprotein (as quantified by the presence of SIA and GAL in the gastric juice) in the stomach of starved control and adrenalectomized animals. SIA usually occupy exposed terminals position on the oligosaccharide chain of glycoproteins (Schauer et al., 2001). In this position, they mask the underlying oligosaccharide residues, e.g. galactose and fucose from degradation by acid and proteolytic enzymes both in solution and on cell surfaces (Kelm and Schauer, 1997). SIA released from the parent glycoprotein are degraded by pyruvate sialate lyase (PSL), yielding pyruvate and the corresponding *N*-acylmannosamine. In this way PSL contribute to the recycling of SIA in animals.

Mucus glycoprotein contains about 13% protein (Scawen and Allen, 1997). Although, results of this study

indicate that protein concentration in the gastric juice varied depending on the treatment group, it is however, not known how much of this protein arise from cellular material or comes from the mucus glycoprotein. On the basis of these observations, the most likely explanation is that adrenal glands seems to be without importance for the secretion of gastric mucus in rats but it may suppress the catabolism of mucin since the activity of PSL was elevated in their absence. Adrenal glands may also play a role in the inhibition of galactosyltransferases or galactosidases as indicated by the slight increase in the concentration of GAL in the gastric juice in their absence. These suggestions may indicate that endogenous glucocorticoids released during stress may not have any influence on the secretion of preformed gastric mucus to resist the harmful actions of ulcerogenic stimuli. Therefore, the protection afforded by glucocorticoids against ulcerogenic stimuli (Filaretova et al., 2001b) may have been through a different mechanism other than increased mucus secretion.

The increased FS and decrease in BS and adherent mucus by reserpine [a dopamine depletory agent] may suggest that this agent facilitate the degradation of mucus without concomitant increase in secretion of preformed mucus from the mucosa granules. This agent also suppresses the activity of PSL, thereby allowing the exposed galactose molecules to be recognized by galactose specific lectins or the immune system which may further worsen the mucosa injury induced by loss of the protective mucus on the gastric mucosa. The result of this study also suggests that the observed action of reserpine on SIA may be mediated via the sympatho-adrenergic system. Evidence for this is seen by the observation that the effect of reserpine on adherent mucus, SIA and PSL was inhibited by prior administration of the -adrenergic blocker, propranolol. Reserpine induced inhibition of PSL and increase in FS was however, not influenced by exclusion of the adrenal glands, suggesting that this response was mainly mediated by direct adrenergic innervations. The action of reserpine may have been elicited from several levels in the central nervous system. However, it is postulated that, the higher brain structures (e.g. hypothalamus) may influence gastric mucin biosynthesis and secretion via the sympatho-adrenergic routes or by yet unidentified hormonal factor and that, the influence is mainly inhibitory. The adrenal glands seem to be without importance in this mechanism.

Gastric mucosal injury induced by adrenalectomy may have been mediated via the glucocorticoid sensitive vascular supply to the stomach as was suggested by Szabo (1984). Additionally, it has been reported that catecholamines stimulate blood flow to the stomach especially to the mucosa and sub mucosa of the gastric antrum (Guth and Leung, 1987). It therefore, appears that the observed gastric protective properties of glucocorticoids (Filaretova et al., 2001b) or catecholamines (Valuenzela et al., 1979) may be mediated through their effects on mucosal blood flow or motility (Filaretova et al., 2002)

and not on mucus secretion.

The picture emerging from these results is that adrenal glands corticosteroids are not required for the degradation or secretion of gastric mucus in rats and that reserpine facilitate the breakdown of gastric mucus and suppress the secretion of preformed mucus probably through the activation of -adrenergic receptors. The gastric mucosal injury observed with corticosteroids administration in earlier studies may be independent on their effect on gastric mucin glycoprotein.

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