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Catecholamines and experimental stress ulcer: morphological and biochemical changes in the gastric mucosa

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SUMMARY

A model for the production of gastric mucosal haemorrhages and ulcers, similar in morphology and distribution to those seen in stress states in man, has been produced by the intraperitoneal injection of adrenaline in single, double or treble doses of 0·6 mg/kg. Lesions were produced in 2 or 3 h, without ligation of the pylorus. Severe mucosal changes were associated with a fall in gastric mucosal histamine concentration. Mucosal serotonin did not change. Rises in mucosal adrenaline were accompanied by a fall in noradrenaline, total catecholamine levels being unchanged.

GASTRIC erosions occur very commonly in 'stress' situations. In one study of 40 severely injured patients, *all* were found to have gastric erosions at endoscopy (1). Severe bleeding from such lesions is not uncommon and carries a mortality of over 50 per cent. Effective therapy is hindered by ignorance of the aetiological factors involved.

Schwarz's dictum (2) 'no acid, no ulcer', which relates to peptic ulcer in general, applies equally well to stress ulceration. Hypersecretion of acid, however, is not usual in stress situations. In such states there may be changes in gastric mucosal permeability, but these are not essential for the development of erosions. In almost all stress states, mucosal ischaemia is an important feature.

The possible aetiological role of catecholamines has not been fully investigated. Intraperitoneal injection of adrenaline has been found to produce gastrointestinal lesions in the dog, cat, rabbit and guinea-pig (3), and the latter two species have been shown to have a mucosal microcirculatory system very similar to that of man (4). It is known that in shock states in man, following myocardial infarction, haemorrhage and sepsis, there is a massive outpouring of catecholamines (5). It is possible that this excessive production of catecholamines may play an aetiological role in the production of erosive gastritis in man.

The present study describes the use of a model for the production of stress ulcer in the rabbit, by the intraperitoneal instillation of adrenaline. Different dose regimens have been compared to assess their effects on the gastric mucosa. Changes in blood pressure and in tissue catecholamines, histamine and serotonin were measured in order to correlate the production of mucosal lesions with biochemical changes in the gastric mucosa.

Materials and methods

Twenty-eight young rabbits, of either sex, weighing between 2·5 and 3·5 kg, were used. The animals were divided into five groups (*Table I*), which were given adrenaline intraperitoneally according to different schedules.

Table I: ADRENALINE ADMINISTRATION REGIMENS

Group (*n*)	Dose (mg/kg)	Time injected (min)	Total dose (mg/kg)	Duration of experiment (min)
A (6)	0·6	0	0·6	120
B (4)	0·6	0·60	1·2	180
C (4)	0·6	0·30	1·2	150
D (10)	0·6	0·30·60	1·8	180
Control (4)	0	—	0	120

For 24 h before commencing each experiment the animals were given only water ad libitum. In spite of this there was some food remaining in the stomach at the end of the experiment in every case. All the animals were killed 2 h after receiving the last intraperitoneal dose of adrenaline (groups A–D), or of saline (control group).

The rabbits were anaesthetized throughout the experiment, a slow intravenous injection of nembutal (Sagatal, May & Baker Ltd, 50–60 mg) being used for induction. The trachea was intubated through a tracheostomy and anaesthesia maintained with 1 per cent fluothane, nitrous oxide (1 l/min) and oxygen (1·5 l/min); the depth of anaesthesia was such as to permit spontaneous respiration.

Under aseptic conditions, the right or left femoral artery was exposed. A No. 14 gauge polyethylene catheter was threaded up to the corresponding iliac artery and secured with a silk ligature. This catheter was attached through a three-way stopcock to a blood pressure recorder (Digital physiological pressure module, Kontron Instruments). The line was flushed periodically with small amounts of heparinized saline (100 units of heparin per 10 ml of saline) to ensure catheter patency and accurate monitoring.

At the end of each experiment the rabbits were given a lethal dose of nembutal. The stomach was removed en bloc with 5 cm of duodenum and both were opened and inspected macroscopically. Photographs and histological sections were made of representative samples. Samples of the glandular portion of the stomach were taken in order to perform measurements of mucosal catecholamines, serotonin and histamine. The intestines were opened and inspected *in situ* and a complete autopsy was performed.

Lesions seen in the stomach were classified as mild—moderate erosive lesions, with no or mild haemorrhage petechiae and/or oedema, but without ulcers—or severe—severe haemorrhagic erosions and/or haemorrhagic ulcers.

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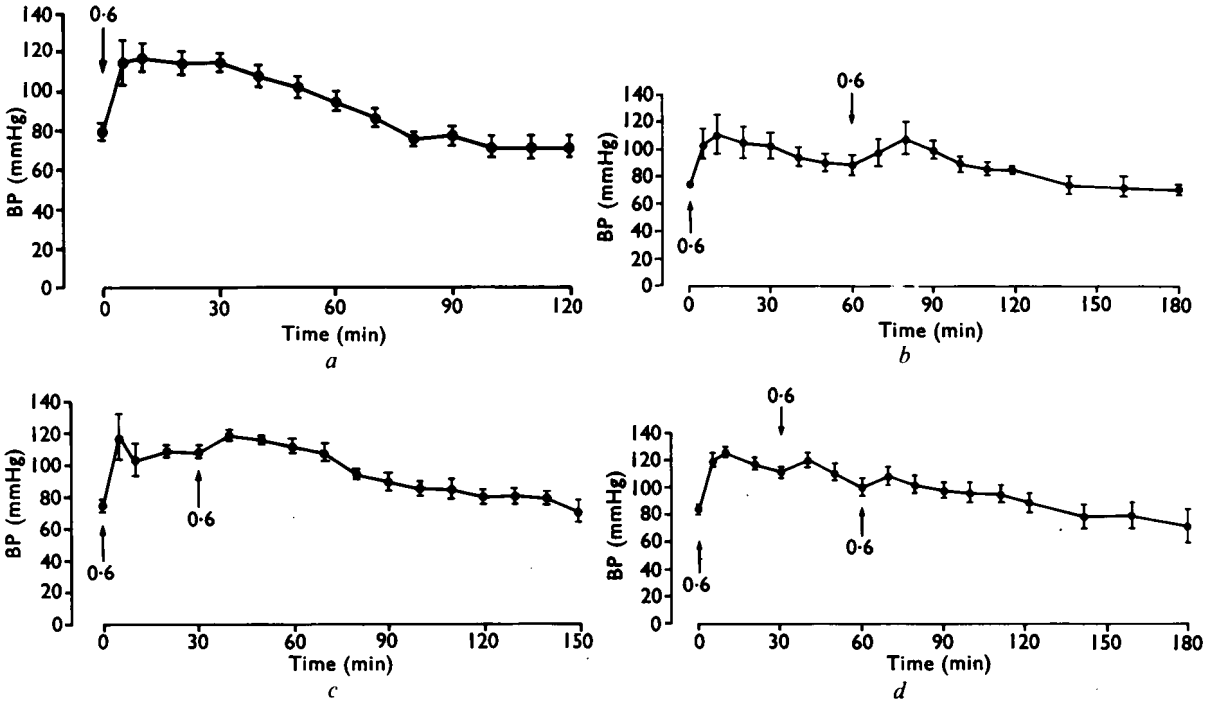

Fig. 1. Changes in blood pressure in response to intraperitoneal adrenaline. Doses of adrenaline (mg/kg) are indicated by arrows.

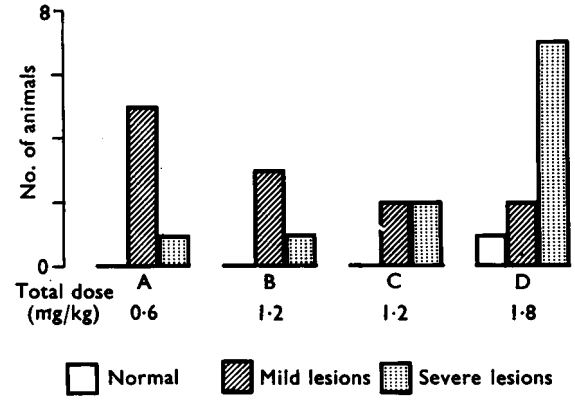

Fig. 2. Lesions produced in response to intraperitoneal adrenaline.

Statistical comparisons were made using Mann–Whitney U tests. P values of $<0{\cdot}05$ are recorded as statistically significant.

Assays of gastric mucosa for catecholamines were based on the method of Campuzano, Wilkerson and Horvath (6), with slight modifications. Serotonin assay was based on a modified method of Weissbach, Waalkes and Udenfriend (7). Histamine was assayed by the method of Hakanson and Ronnberg (8).

Results

Physiological and gross mucosal changes

Control: In the control group 4 rabbits received 5 ml of normal saline intraperitoneally; no adrenaline was given. Blood pressure remained unchanged throughout the experiment. All the rabbits in this group survived, until killed at 120 min. Autopsy revealed no lesions in the stomach, duodenum, antrum or any other viscera, abdominal or thoracic.

Group A: This group of 6 rabbits received a single initial dose of adrenaline. Changes in systolic blood pressure (BP) are represented in *Fig.* 1*a*. Blood pressure rose from $79 \pm 3{\cdot}6$ mmHg at 0 min to $118 \pm 6{\cdot}3$ at 15 min, then fell progressively to normal ($72 \pm 5{\cdot}1$) at 120 min. All the rabbits in this group survived until the end of the experiment.

Macroscopic examination revealed gastric lesions in all rabbits, mild in 5 and severe in one (*Fig.* 2). In the single rabbit with severe lesions a greater acidosis had been noted. Gastric lesions occurred in the fundus and body, the gastric antrum being free of lesions; in only one case were some prepyloric haemorrhagic erosions seen. All the rabbits showed bilateral haemorrhagic congestion of the lung bases.

Group B: This group of 4 received two 0·6 mg/kg doses of adrenaline, 1 h apart. Systolic blood pressure (*Fig.* 1*b*) showed a rapid increase during the first 10 min (from $74 \pm 1{\cdot}3$ mmHg to $110 \pm 14{\cdot}1$), then fell progressively until 60 min, reaching $88 \pm 7{\cdot}0$. It rose again after the second adrenaline injection and then fell progressively once again until 180 min (mean $53 \pm 17{\cdot}9$ mmHg).

In this group, 1 rabbit died at 170 min and the remainder survived. On macroscopic examination, 3 rabbits had mild gastric lesions and 1 a severe lesion (*Fig.* 2). In all cases the gastric lesions were located in the fundus and cardia. Both antrum and duodenum were free of lesions. Autopsy did not reveal any other important lesions. Total levels of catecholamines in the gastric mucosa were not significantly different from those in controls, though the level of adrenaline was

Table II: ASSAYS OF GASTRIC MUCOSA

Group (*n*)	Total catecholamines (nmol/g wet)	Adrenaline (nmol/g wet)	Noradrenaline (nmol/g wet)	Histamine (nmol/g wet)	Serotonin (nmol/g wet)
A (5)	2·298 ± 0·413	1·297 ± 0·205	1·001 ± 0·483	39·49 ± 10·19	26·90 ± 8·29
B (4)	4·887 ± 1·595	4·195 ± 0·183	0·693 ± 0·183	39·30 ± 16·54	47·32 ± 20·62
C (4)	3·186 ± 0·506	2·580 ± 0·406	0·607 ± 0·189	36·66 ± 8·82	31·90 ± 19·90
D (9)	3·121 ± 0·385	2·845 ± 0·415	0·276 ± 0·086	74·04 ± 11·36	35·16 ± 8·80
Control (4)	3·532 ± 0·323	0·125 ± 0·018	0·408 ± 0·385	81·46 ± 24·48	55·14 ± 20·13

significantly higher and that of noradrenaline significantly lower.

Group C: This group of 4 received two 0·6 mg/kg doses of adrenaline, 30 min apart. Systolic blood pressure rose rapidly during the first 5 min (from 74 ± 3 mmHg to 118 ± 13·5), then fell slowly until 30 min (*Fig.* 1*c*). There was a rise after the second adrenaline injection followed by a fall to initial values at the end of the experiment. All the rabbits survived until the end and were killed at 150 min, that is, 120 min after the last dose of adrenaline.

On macroscopic study, all the rabbits presented gastric lesions, located in the fundus and on the greater curvature of the stomach. These lesions were considered mild in 2 and severe in another 2 (*Fig.* 2). The antrum and duodenum were normal and autopsy revealed no other lesions. The mucosal level of adrenaline was significantly higher than in the control group and the level of noradrenaline was significantly lower.

Group D: This group of 10 rabbits received three 0·6 mg/kg doses of adrenaline. Systolic blood pressure (*Fig.* 1*d*) showed a rapid increase in the first 15 min, rising from an initial 83 ± 3 Hg to 122 ± 3·5 at 15 min. This was the highest blood pressure reached in any of the groups. It then fell progressively, apart from brief rises after subsequent doses of adrenaline, to 80 ± 10·5 at 120 min and 29 ± 12·6 at 180 min. In the 150-min and 180-min samples blood pressure was significantly below initial values. In this group, 9 of the 10 animals survived for 120 min, 7 for 150 min and only 4 survived 180 min. Thus, half of the rabbits died between 120 and 180 min.

In the macroscopic study, 9 animals had gastric lesions, 2 of which were mild and 7 severe. The other rabbit showed no lesions (*Fig.* 2). This group had the greatest incidence of severe lesions. The lesions occurred principally in the fundus and body. Seven animals presented a normal antrum and 3 had superficial antral erosions. Half of the animals showed severe haemorrhagic lesions in the duodenum and all had bilateral haemorrhagic congestion of the lung bases. Mucosal levels of adrenaline were significantly higher than in controls and levels of noradrenaline were significantly lower; the level of total catecholamines in the mucosa was not significantly different.

Mucosal catecholamines

The mucosal levels of adrenaline and noradrenaline at the end of the experiments are indicated in *Table II*. Total catecholamines did not differ significantly from control values in any group. As expected, after injection of adrenaline mucosal levels of adrenaline rose significantly and this was associated with a significant fall in the mucosal levels of noradrenaline.

Mucosal serotonin

In no group did the mucosal serotonin level at the end of the experiment differ significantly from that in controls (*Table II*).

Table III: MUCOSAL HISTAMINE AND GASTRIC LESIONS

Lesions (*n*)	Histamine (nmol/g wet) (mean ± s.e.mean)
None (5)	88·59 ± 18·12*
Mild lesions (11)	54·11 ± 10·42
Severe lesions (10)	47·99 ± 6·15*

* Difference between the means was statistically significant ($P = 0·01$).

Mucosal histamine

The histamine levels in the gastric mucosa at the end of the experiments are tabulated in *Table II*. In no group did the mean value differ significantly from that in the control group. When the data were assessed independently of groups, however, and divided according to the existence of no, mild or severe lesions, as in *Table III*, it was found that the more severe lesions were associated with a significantly lower mucosal histamine. No such association was observed with the mucosal serotonin values.

Histology

The histological changes found were bland and no inflammatory reaction or thrombosis of vessels was seen. The minimal change was desquamation of the surface epithelium, which in many sections remained trapped in a surface layer of mucus. The second, more severe, change recognized was the loss of epithelium lining the gastric pits. At this stage the apparent thickness of the mucosa was slightly reduced and the more superficial cells of the gastric glands stained less intensely and had become rounded, somewhat shrunken and hyaline in appearance (seemingly losing cohesion). More advanced changes than these decreased the mucosal thickness still further with corresponding changes in the more superficial gland cells. The loose fibrous tissue strands of the lamina propria had a hyaline, palely staining appearance similar to the gastric gland cells and in both this may represent the effect of peptic digestion. The haemorrhages in the lamina propria occupied the upper middle or lower thirds with no consistent pattern.

In the duodenum the changes were analogous, beginning with loss of villous surface epithelium followed by disintegration of the connective tissue core of the villi. No erosion penetrated the muscularis mucosa.

Although there were obvious differences in the macroscopic changes in the various groups, it was not possible to correlate this with histological grading of the mucosal lesions. The major problem was the presence of artefacts. All animals, although fasted, were found to have food residue in their stomachs. This was washed away, before macroscopic examination, without producing visible changes. At microscopy, however,

superficial changes were present in all groups, including the controls. This aspect is the subject of another current study.

Discussion

Other workers have developed experimental models to study the possible aetiological role of catecholamines in stress ulcer. Penner and Bernheim in 1939 (3) gave daily intraperitoneal injections of adrenaline, in doses varying between 1·2 and 10 mg/kg, to dogs, cats, guinea-pigs and rabbits. One rabbit developed a gastric ulcer. They found that rabbits and guinea-pigs produced lesions similar to those seen in stress in man, beginning with submucosal congestion and oedema and going on to frank haemorrhage. Klemperer et al. (4) discussed these results and emphasized that rabbits and guinea-pigs had a mucosal circulation dissimilar to that of the cat and dog, but very similar to that of man. Baronofsky and Wangensteen (9) found that injections of histamine alone produced no ulceration in the rabbit, though histamine with adrenaline produced stress ulcers. Adrenaline alone was not used in their studies in the rabbit, though they found that adrenaline in bees wax produced gastric erosions (plus one duodenal ulcer) in dogs. Following along similar lines, Nicoloff et al. (10, 11) found that if adrenaline was injected into the left gastric artery during the stimulation of acid secretion, localized ulceration of the mucosa could be produced. In 1964 the same workers were able to show that infusion of either adrenaline or noradrenaline in dogs reduced both acid secretion and mucosal blood flow. Sethbhakdi et al. (12) gave intraperitoneal adrenaline, 0·4 mg/kg, after ligating the pylorus of rabbits. In every rabbit this produced ulcers that had distinctive features. First, they all occurred in the acid-secreting 'fundus' and not in the antrum, in contrast to findings in the Shay rat preparation. Secondly, the ulcers were reproducibly severe. Thirdly, they were acid-dependent. Antacids greatly reduced the severity of ulceration and after truncal vagotomy ulcers could not be produced. These authors found that adrenaline produced more severe changes than noradrenaline given in the same manner. Later in 1970 (13) they reported that after vagotomy, if acid was infused into the stomach, ulcers could once more be produced. In the same model they produced evidence that adrenergic blockers may prevent the formation of ulcers and that histamine antagonists also diminished this process.

In the present study it was found that the intraperitoneal injection of adrenaline produced stress lesions similar to those described by Sethbhakdi et al. (12). As expected, adrenaline consistently produced a rise in blood pressure, followed by a profound fall when the largest doses were given. Stress ulcers and intramucosal haemorrhages were seen commonly in the animals given adrenaline, but never in the controls. This was accomplished in a short space of time (never more than 3 h) and without ligation of the pylorus. We have found in further pilot experiments that *intravenous* infusion of adrenaline produces severe gastritis without discrete ulceration. The reason for this different response is being investigated. It may be that intraperitoneal injection, as used originally by Penner and Bernheim (3), giving high 'pulses' off circulating adrenaline, may trigger off mucosal disruption similar to that produced in short periods of haemorrhagic shock, as reported by Skillman et al. (14).

Initial macroscopic examination indicated the presence of mild to severe haemorrhages, small superficial erosions and, in the more severe cases, haemorrhagic ulcers.

The histological changes seen were consistent with the very short duration of the experiments and varied greatly in their intensity. The poor correlation with the macroscopic changes seen on opening the stomach, which were pronounced and easily recorded by colour photography, was disappointing and almost certainly due to artefacts induced in clearing the stomach of food residue. This difficulty has caused us to alter our technique in subsequent experiments in which the pathological processes are being examined more precisely.

The lesions produced, as observed both macroscopically and microscopically, were strikingly similar in nature and distribution to the lesions found in stress states in man.

Although variations in circulating levels of serotonin have been proposed as a possible factor in the aetiology of stress ulcers, no significant changes occurred in gastric mucosal serotonin in these studies. The significance of this observation is uncertain at the present time. The changes seen in gastric mucosal catecholamines were consistent with the take-up of injected adrenaline. Their role in the aetiology of the mucosal lesions is matter for conjecture at the moment, but may possibly be very relevant to the stress situation in man.

In shock states in man, plasma catecholamine levels are raised. This is mainly a neurogenic outpouring of adrenaline, mediated in part by the moderator reflexes (15). A decrease in blood flow through the adrenal medulla may also stimulate secretion.

It has also been postulated that the reactivity of vessels to such circulating adrenaline may be exaggerated in the presence of endotoxin. It may well be that the increased circulating adrenaline in shock states may contribute to the magnitude of the tissue ischaemia seen.

Mucosal histamine was measured in the stomach in these studies. Insufficient work has been done in this field to interpret the results fully. The basic finding that severe mucosal lesions were associated with depleted mucosal histamine was of interest. It has been found that mucosal histamine is lower in duodenal ulcer subjects, who secrete more acid, than in control subjects (16). Isolated measurements of histamine, however, give no insight into the dynamic situation in the mucosa and further exploration of this field is clearly indicated.

In the present model the injection of adrenaline produced both a shock state and gastric mucosal changes consistent with ischaemia; the appearance and distribution of these mucosal lesions was strikingly similar to those seen in man. In view of the known similarity in microvascular anatomy between rabbit and man, further more detailed study of the mechanisms involved in the production of such lesions may be of direct clinical relevance. Certainly the pattern of production of lesions in this animal situation seems much more analogous to the human situation than that which is seen after prolonged stress or ligation of the pylorus in other models frequently studied. As the prevention and treatment of stress bleeding remains an important and unsolved clinical problem, we suggest that this new animal model may be of value in further elucidation of aetiological mechanisms and in the study of therapeutic agents.

Further study must include evaluation of other

important factors such as acid secretion and mucosal blood flow.

Acknowledgements

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Against multiple choice questions

Sir George Pickering argues the case against multiple choice questions in reply to an article which appeared in *Medical Teacher* 1979; **1**: 37–42.

The respect that is engendered in the student for dogma terrifies me. Are we witnessing a return to authoritarianism and the tyranny of opinion such as suppressed intellectual freedom in the late Middle Ages? Is history going to repeat itself and are we on the threshold of another Dark Age? The kind of knowledge that they test is that which a Dean of Harvard had in mind when he said 'I tell my students that one of the difficulties they will face in medicine is that in 10 years' time they will find that half of what they were taught is wrong and neither I nor any of my teachers know which half.'

Medical Teacher 1979; **1**: 850.