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Activation of pepsinogen by sulphated glycosaminoglycans: a possible role in peptic ulcerogenesis

Sulphated glycosaminoglycans (SGS) occur in the gastrointestinal tract at lumenal and intracellular levels, and it has been suggested that they may be secreted by gastric zymogen cells (Schrager, 1964; Spicer, 1965; Spicer & Sun, 1967; Gerald, de Graeff & others, 1967). This association of SG and the source of gastric pepsin has led to the suggestion of an antipeptic role for SG at intracellular level (Gerald & others, 1967). Our experiments now suggest that a more subtle relation may exist between the zymogen pepsinogen and SGS.

Back diffusion of hydrogen ion in the abnormally permeable gastric mucosa present in gastric ulcer has been shown (Davenport, 1965; Overholt & Pollard, 1968); there is therefore a distinct possibility that at least regions of specific gastric cells may attain an abnormally low pH. With the chief cell, absorption of hydrogen ion would give rise to a system containing pepsinogen, hydrogen ion and SG. While the intracellular concentration of hydrogen ion may not reach a level sufficient to activate the pepsinogen at a significant rate, any marked increase in the rate of activation could, in conjunction with the autocatalytic nature of the acid activation of pepsinogen (Herriott, 1938), cause significant pepsin production and possibly consequent intracellular proteolysis.

Pepsinogen (Sigma Chemical Co.) was activated at various pH values and constant ionic strength for varying periods of time in the presence and absence of the SGS chondroitin sulphate-A or heparin, and the resulting pepsin was destroyed by raising the pH to 8. The pepsinogen remaining (unactivated) was measured by rapid activation at pH 1·6 followed by assay of the peptic activity using the haemoglobin digestion method. This technique (after Herriott, 1938) allows the effect of the SGS on the activation of pepsinogen, determined by difference, to be observed.

Pepsinogen (10 mg) was dissolved in 0·002 M phosphate buffer pH 6·9 (25 ml), and the varying amounts of SG added* to this solution which was kept at 37° for 10–15 min. Acetate buffer (9 ml) (Long, 1961), ionic strength 0·05, at the pH of activation (pH 3·6, 4·0, 4·3) and 37° was added at zero time to start activation. Aliquots (2 ml) were removed at suitable time intervals, mixed with saturated sodium tetraborate solution (2 ml) which had been diluted so that the pH was thereby raised to 8–8·5, and allowed

* Heparin 0·2–5 mg; chondroitin sulphate 1–20 mg.

to stand 10 min. The pepsin thus destroyed, the remaining pepsinogen was activated by adding 1 ml HCl solution (to adjust pH to 1–2) and the mixture allowed to stand 5 min (solution A). Peptic activity was determined by adding 1 ml of solution A to 5 ml haemoglobin (Armour Laboratories Ltd.) solution at pH 1·6 and measuring the digestion (Northrop, Kunitz & Herriott, 1948). Extinctions were read at 280 nm within 15 min of dilution and units calculated by reference to a standard curve for crystalline pepsin.

From plots of percent pepsinogen remaining *vs* time, the time for 50% activation of pepsinogen was calculated (t50%); the amount of SG required to halve this time was also determined graphically. The results in Table 1 show that this was a fraction of the weight of pepsinogen used.

Table 1. *Ratio of weight of sulphated glycosaminoglycan: weight of pepsinogen which halves the t50% for pepsinogen*

pH of activation	t50% for pepsinogen min mean ± s.d.	Ratio for ½ t50%	
		CHS / Pg	H / Pg
3·6	15 ± 2·3	0·18; 0·20; 0·20	0·10; 0·08; 0·11
4·0	51 ± 3·6	0·19; 0·17; 0·20	0·11; 0·12; 0·10
4·3	170 ± 6·4	0·22; 0·19; 0·21	0·07; 0·13; 0·09

CHS = chondroitin sulphate; H = heparin; Pg = pepsinogen; t50% = time for 50% activation of pepsinogen at the stated pH in the conditions of the experiment.

Pepsinogen activation occurs below pH 5 with release of inhibitor (Herriott, 1938, 1941), thus:

$$\text{pepsinogen} \rightarrow \text{pepsin} + \text{inhibitor} + \text{peptides}$$

Even in excess quantity, SGs did not activate pepsinogen above pH 5 in our experiments, indicating that a certain minimum hydrogen ion concentration is required for any activation to occur. Neither SG altered the pH of the solutions.

The results show that chondroitin sulphate-A halved the t50% for five times its weight of pepsinogen, and heparin for ten times its weight, between pH 3·6 and 4·3. Interaction of SG with the cationic inhibitor released during activation is suggested as the mechanism by which SGs accelerate the activation of pepsinogen.

Three points arise. First, if a cell which normally contains pepsinogen and SG absorbs hydrogen ion so that the pH falls below 5, SG will accelerate zymogen activation. Since in most cells a fall to well below pH 5 is unlikely in the presence of some cell integrity, acceleration of activation would assume importance.

Second, this action of SG at very low concentration must be clearly distinguished from the antipeptic activity of sulphated polysaccharides now known to be effected by substrate protection (Anderson & Baillie, 1967).

Third, pepsinogen occurs in the chief cells of the stomach principally encapsulated in granules. These coacervate-like globules will be disrupted by any acid which is absorbed, liberating pepsingoen. Consequent zymogen activation will be speeded up by the free SG in the cell even in the presence of acid sufficient only to lower the pH to just below 5. Thus intracellular proteolytic potential is increased by the presence of SG when small quantities of acid are absorbed. An ulcerogenic tendency of the intracellular pepsin produced is a reasonable assumption. This "pathological" action of SGs would be of most consequence when restricted quantities were present; when excess was available the (normal) antipeptic action of SGs would offset the effects of accelerated pepsin production. Hakkinen (1966) has pointed out that mucosal sulphate is depleted when ulceration occurs.

This concept may help to relate the antisecretory (Anderson, Marcus & Watt, 1962; Eagleton, Watt & Marcus, 1968) and antipeptic actions of SGS in respect of their anti-ulcer action.

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Amphetamine-induced release of [^{3}H]metaraminol from subcellular fractions of the mouse heart

The mode of action of amphetamine is complex and there are many studies reporting various sites of attack for this drug.

Amphetamine has a cell membrane pump blocking action—but it is not as potent as for example protriptyline (Carlsson & Waldeck, 1965; Malmfors, 1965; Carlsson, Lindqvist & others, 1965). It has also been suggested that amphetamine in large doses has a direct releasing effect on the amine storing granules. More recently Carlsson, Lindqvist, & others (1966a) suggested that amphetamine in low doses might act by displacement of amines from hypothetical extragranular binding sites or by an effect on the cell membrane leading to increased release (Carlsson, Fuxe & others, 1966b).

The aim with the present work was to see if studies on subcellular amine distribution could further elucidate the mode of action of amphetamine. This approach has proved useful for clarifying the mechanism of action of other drugs influencing adrenergic mechanisms (Lundborg, 1967). As in much of our previous work [^{3}H]-metaraminol, a noradrenaline analogue resistant to both monoamine oxidase and catechol-*O*-methyl transferase, was used.

Mice in groups of six, were given [^{3}H]metaraminol, 0·04 mg/kg, intravenously. Control groups received no further treatment and were killed by decapitation 30 min later. Other groups were injected with (+)-amphetamine bitartrate 5, 1 or 0·2 mg/kg (calculated as the salt) 15 min after the [^{3}H]metaraminol administration and were killed 15 min later. All animals were kept at an ambient temperature of 30°.