noted are secondary to the caloric effect of the hormone, remains as an open question.

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Plasma motilin levels in duodenal ulcer and effect of a truncal vagotomy and hypoglycaemia

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Summary. The identical response of plasma motilin levels, in duodenal ulcer patients and healthy controls, to a test meal and insulin induced hypoglycaemia, fail to demonstrate any significant abnormalities in motilin release. The close correlation between blood glucose and motilin suggest a possible role of this new hormone in carbohydrate metabolism.

The mechanisms that control the levels of plasma motilin have not been elucidated. Recently it has been demonstrated in man that instillation of acid into the duodenum results in a sharp rise of plasma motilin[2]. Infusions of exogenous motilin at concentrations within the physiological range have been reported to produce a significant delay in gastric emptying in heathly volunteers[3]. The enhanced rate of gastric emptying and increased gastric acid production in patients with duodenal ulcer led us to study the effects of a test meal and also of insulin hypoglycaemia on plasma motilin in such subjects.

Patients and methods. A previously described sensitive radioimmunoassay specific for motilin was used[4] which was able to detect changes in plasma motilin of 3 pmoles/l with 95% confidence. Antibodies were raised to pure porcine motilin and used at a final dilution of 1 in 240,000 bound approximately 50% of the added I^{125} motilin. No cross reaction was observed on addition of 1 µg/tube of the other available intestinal hormones or with 100 ng/tube of the synthetic fragments of motilin (1-6, 7-22, 12-22). Gel chromatography of human gut extracts showed only a single major peak of motilin immunoreactivity eluting in the identical position to pure porcine motilin[4]. Blood samples were taken with 10 units of heparin, rapidly separated and the plasma frozen within 15 min of collection. Plasma motilin immunoreactivity was measured after an overnight fast in controls and patients in response to 2 types of stimuli. Blood glucose was measured by glucose oxidase[5] method.

1. Test meal: A standard breakfast of eggs and toast composed of 66 g of carbohydrate, 18 g of protein and 22 g of fat was given after an overnight fast to 16 healthy controls aged 27-64 (mean 41) and 10 patients with an active duodenal ulcer proven by endoscopy or barium meal aged 29-63 (mean 39).

2. Insulin induced hypoglycaemia: A 2nd group of 6 controls, 16 duodenal ulcer patients and 8 patients following complete truncal vagotomy (Holanda -ve) received an i.v. injection of insulin (0.2 units/kg). Prior to the test a Ryles tube was inserted and gastric juice was drained throughout and discarded.

Calculations: Motilin concentrations are expressed both in pmoles/l and, in order to overcome the considerable variation in basal levels from individual, as percentage increments. Differences were examined parametrically (e.g. t-test) for percentage change and nonparametrically (e.g. Wilcoxon sum of ranks tests) for absolute values.

Results. Figure 1 shows the basal plasma motilin in the 3 groups. A considerable individual variation is seen. The median concentration in the controls is 33 pmoles/l (range 10-110), all duodenal ulcer patients 42 pmoles/l (range 8-258) and patients following complete truncal vagotomy 48 pmoles/l (range 15-316). The differences are not statistically significant.

Figure 2 shows the effect of a standard meal on plasma motilin levels in the group of 16 control subjects and 10 duodenal ulcer patients. In the control group a 15 pmoles/l (range 3-50) incremental rise of plasma motilin was observed 15 min following ingestion of the meal, representing a mean individual percentage increment of $32.5 + 12\%$ ($p < 0.02$). An identical rise was also observed in the duodenal ulcer group. In controls motilin levels then fell below basal reaching statistical significance at 90 and 135 min ($p < 0.02$), whereas in DU patients they only fell slightly after the initial peak and stayed thereafter at approximately fasting values. At no point during the experiment did the motilin concentrations in the DU patients differ significantly from the controls.

Following insulin-induced hypoglycaemia motilin levels fell sharply (figure 3) reaching a nadir at 35 min (a fall of $58 \pm 8\%$ in controls ($p < 0.005$) and $42 \pm 3\%$ in DU ($p < 0.001$), which coincided with the lowest blood glucose

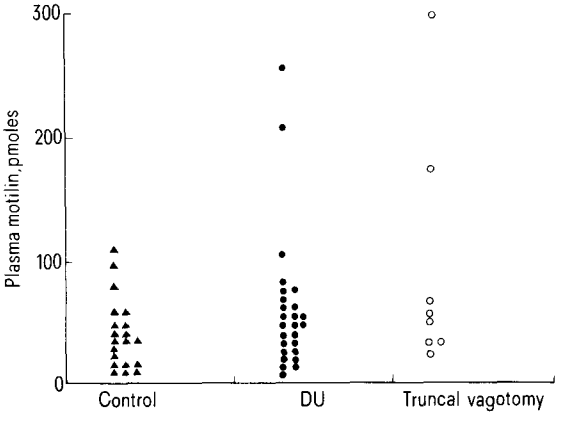

Fig. 1. Basal plasma motilin in 26 active duodenal ulcer patients, 8 post-vagotomy patients and 20 normal volunteers.

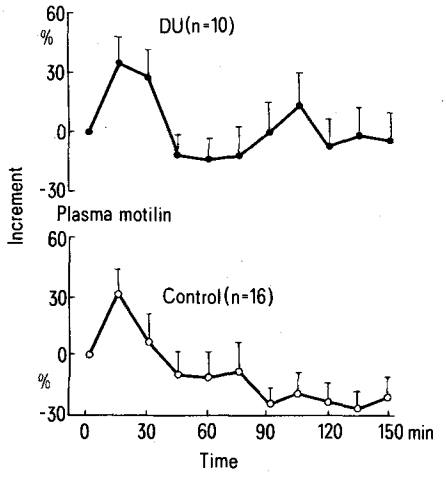

Fig. 2. Percentage increment in plasma motilin in 10 active duodenal ulcer patients (top graph) and 16 normal volunteer (bottom graph) following a standard hospital lunch.

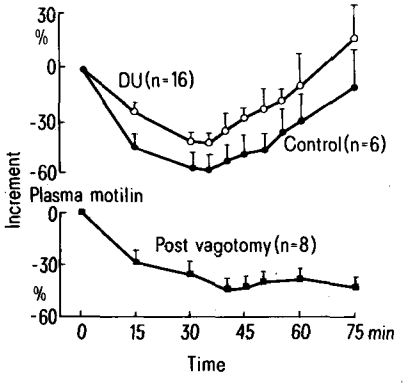

Fig. 3. Percentage increment in plasma motilin in 16 active duodenal ulcer patients and 6 normal volunteers (top graph) and 8 post-vagotomy patients following insulin hypoglycaemia.

levels (mean glucose in both groups 2.1±0.1 mmoles/l at 35 min). Plasma motilin also fell in the 8 post-vagotomy patients, but levels remained significantly depressed for longer 42±5% at 75 min ($p < 0.001$), probably due to the slower recovery of plasma glucose concentrations in this group.

The integrated percentage motilin decrement between 0 and 75 min for each individual was compared with the integrated percentage glucose decrement for the same period (figure 4). A highly significant correlation was found ($r = 0.87$, $n = 27$, $p < 0.001$).

Discussion. Motilin was first discovered because of its potent effects on motor activity in the upper gastrointestinal tract[6,7]. In man it is found in high concentration in the duodenum, jejunum and upper ileum where it is produced by a specific sub-population of the mucosal endocrine enterochromaffin cells[8]. The measurement of human plasma motilin is not technically difficult as it circulates in relatively high concentrations and appears to exist in only a single molecular form. It does not have sequence similarities with any other known hormone[4]. In spite of this, progress in documenting its physiology in man has been slow, perhaps because of shortage of the pure hormone. It is already clear that large species differences exist, for example in the pharmacology of motilin. The antiserum

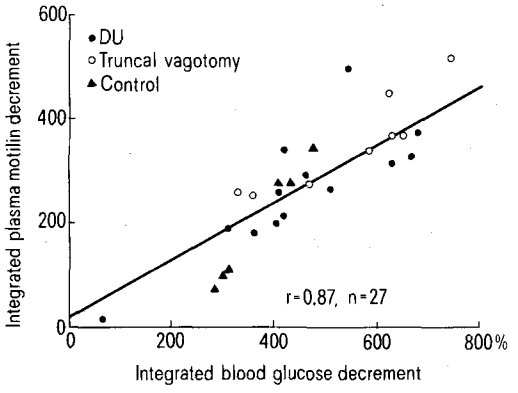

Fig. 4. Relationship of the integrated percent plasma motilin decrement and the integrated percent blood glucose decrement, following insulin-induced hypoglycaemia in 13 duodenal ulcer patients, 8 post-vagotomy patients and 6 normal controls.

used in this study does not cross react with canine motilin[9] suggesting a significant structural difference. No antibody has yet been found, however, which can distinguish between porcine and human motilin. As motilin from these 2 species also shows identical physico-chemical behaviour it seems likely that they are closely similar and thus radioimmunoassays based on porcine motilin will yield valid information concerning the human hormone.

Infusions of motilin in man at concentrations which appear near physiological, increases lower oesophageal sphincter tone[10], delays gastric emptying[3] and decreases small intestinal transit rate[11]. On the other hand no significant effect on salivary, gastric or pancreatic secretions was noted[12]. The only agent so far shown to cause release of motilin in man is the intraduodenal infusion of strong acid[2]. Smaller and possibly more physiological, quantities of acid do not, however, cause significant release (unpublished observations) and thus this may not be an important physiological control mechanism. The location of motilin in the upper small intestine implies a possible role in the post digestive processes. We report here a statistically significant rise in motilin in the early postprandial phase which, however, is not sustained. This finding may be construed as against an important role for motilin as a hormone controlling the fasting interdigestive myoelectric complex in man[13]. The rate of gastric emptying is clearly one of the factors controlling duodenal pH. It is known to be abnormally increased in patients suffering from duodenal ulcer[14]. It therefore was important to investigate any possible abnormality of motilin release in DU patients but it appears that neither basal motilin, its release by food or suppression by hypoglycaemia differs significantly from normal controls.

The close correlation between the fall of motilin and glucose following insulin was unexpected. Administration of glucose either i.v. or orally does not cause motilin release[15]. Therefore it seems likely that the relationship of motilin to glucose during hypoglycaemia is indirect. A successful truncal vagotomy did not affect the motilin decline and provides evidence that the role of the vagus in controlling motilin release may be unimportant. Vagal interruption blocks the release of PP[16] and greatly reduces gastric acid and pancreatic secretion, thus suggesting that these factors are not mediators of the motilin inhibition.

The distribution of motilin almost exactly parallels that of GIP (glucose-dependent insulin-releasing peptide)[17]. It seems likely that GIP provides a major mechanism for the gastrointestinal tract to influence metabolism[18]. The possibility that a similar role may exist for motilin requires investigation.

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The effect of methallibure (I.C.I. 33, 828) on the steroidogenesis in the ovary and testis of a fresh water teleost, *Cyprinus carpio*

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Summary. Methallibure treatment of mature *Cyprinus carpio* causes a marked reduction in the steroidogenesis, as indicated by the fall in 3β-HSD activity in the gonads. Significance of these results is discussed.

The surgical hypophysectomy and replacement therapy remain the most convincing experimental procedures for the demonstration of pituitary regulation of gonadal functions. Hypophysectomy is not only technically difficult, but it also disrupts the entire endocrine system. For the specific gonadotrophin supression, several compounds have been used, most of which are steroids which invariably interfere with feedback pathways[2]. In recent times several workers have advocated the use of a nonsteroidal antigonadotrophic compound (methallibure; I.C.I. 33, 828) in place of surgical hypophysectomy[3-12]. Wiebe[4] and Van Ree[10] reported a reduction in the level of histochemically demonstrable steroid dehydrogenases in the gonads as a result of methallibure treatment. The present study deals with the effect of methallibure treatment on the activity of Δ_5-3β-hydroxysteroid dehydrogenase (3β-HSD) in the gonads of fish, *Cyprinus carpio*.

66 mature specimens (33 female and 33 male) of *Cyprinus carpio* measuring 25-30 cm and weighing 100-120 g, were divided into 11 separate groups (each group consisting of 3 female and 3 male specimens), and housed in 300 l aquaria, which were kept aerated periodically. No feeding was done during the experimental period, which lasted 35 days. A suspension containing 1.0 g methallibure/100 ml distilled water was prepared and maintained with Tween 80 (2 drops/10 ml). Groups 1-5 were given 3.0 ml dose, and groups 6-10, 5.0 ml dose, on alternate days. The suspension was dissolved in the aquarium water. The experiments were timed in such a way that 10th day of groups 1 and 6, 20th day of groups 2 and 7, 25th day of groups 3 and 8, 30th day of groups 4 and 9, and 35th day of groups 5 and 10, fell on the same day. Group 11 served as control. The procedure regarding the determination of enzyme activity has already been reported[13]. The only modification made was that, instead of the mixture of propylene glycol and dimethyl formamide (1:1), the dehydroepiandrosterone (DHA) was dissolved in dimethyl formamide. The relative differences in the enzyme activity between the control (group 11) and various experimental groups (1-10), and among the various experimental groups (1-5, and 6-10), were worked out and the Student t-test was applied to the data for comparing the effect of one exposure period and/or the concentration of the chemical, with the others.

Results and discussion. The table summarizes the results of the experiments. It can be seen that mathallibure treatment caused a time-dependent reduction in the activity of 3β-HSD, both in the ovary and the testis of *Cyprinus carpio*. The differences with regard to the enzyme activity between the control and various treated groups (1-10) are highly significant ($p < 1\%$). There is also a significant difference ($p < 5\%$) in the enzyme activity among the different groups (1-5), and (6-10). The differences within each group were insignificant. Also, when the enzyme activity between the 2 groups treated for the same period was compared with regard to the effect of concentration of methallibure, the difference were found to be statistically insignificant. This may probably indicate (though within a limited range of dosages used) that the time interval may be critical for the proper effect of methallibure on the steroidogenesis in fish gonads.

It may be mentioned that the differences in enzyme activity between the groups exposed to 35 day and 40 day treatment (at both concentrations) were insignificant and have not been recorded in the table.

The level of 3β-HSD, along with that of various other steroid dehydrogenases, is indicative of steroidogenesis in the gonadal tissue (see ref. 14 for various criteria of steroidogenesis in a tissue). Hypophysectomy in animals causes a reduction in the histochemically demonstrable level of 3β-HSD[15]. The fall in the histochemically demonstrable[4,10] and biochemically demonstrable level of 3β-HSD (as observed during the present studies), may be because of the action of methallibure at gonadotrophin level[4,10,12]. The exogenous gonadotrophin treatment in case of methallibure-treated fish/hypophysectomized animals, stimulates the activity of steroids dehydrogenases[16-20]. The gonadotrophins are also known to stimulate cyclic AMP synthesis[21-24] (via adenyl cylase stimulation)[21], and also RNA and protein synthesis[25-30]. The action of methallibure, as related to the steroidogenesis in the gonadal tissue, may