SUPPRESSIBILITY OF PLASMA MOTILIN BY ORALLY ADMINISTERED GLUCOSE IN PATIENTS WITH HYPERTHYROIDISM AND LIVER CIRRHOSIS

YUTAKA SEINO, KOZABURO MORI, SUSUMU SEINO, YOSHIKATSU MIYAMOTO*, NOBORU YANAIHARA** and HIROO IMURA

Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto, *Amagasaki Hospital, Amagasaki, and **Laboratory of Bio-organic Chemistry, Shizuoka College of Pharmaceutical Science, Shizuoka, Japan

ABSTRACT

Plasma motilin levels were investigated in normal subjects and patients with hyperthyroidism and liver cirrhosis by dextran-coated charcoal radioimmunoassay using a guinea pig antiserum raised against synthetic motilin. Fasting plasma motilin levels in normal subjects were widely distributed, ranging from less than minimal detectable level (50 pg/ml) to 485 pg/ml, with a mean (± SE) level of 224 ± 36 pg/ml.

Patients with hyperthyroidism and liver cirrhosis also had widely scattered fasting motilin levels of 314 ± 56 pg/ml and 391 ± 71 pg/ml, respectively. Following 50 g oral glucose loading, plasma motilin levels gradually decreased in both groups. These results suggest that plasma motilin is suppressed by oral glucose loading in hyperthyroidism and liver cirrhosis.

KEY WORDS motilin / oral glucose loading / hyperthyroidism / liver cirrhosis

Motilin, a novel hormone extracted from the small intestine, is very distinct in biochemical and physiological characteristics from other gastrointestinal hormones. Brown et al. succeeded in isolating motilin and proposed its amino acid sequence (3, 19). Wünsch et al. (23) synthesized a motilin analog, 13-norleucine motilin, which has been demonstrated to have a contraction-promoting effect on the smooth muscle of the gastric antrum, duodenum and colon, as does natural purified motilin. More recently, Yajima et al. (22) and Shimizu et al. (21) synthesized motilin composed of the whole amino acid sequence. These synthetic motilins have shown biological activity identical to natural hormone (21, 22). However, very few studies have been done on the regulation of its release. Recently, we have demonstrated that plasma motilin fluctuates in the fasting state in man (12) and dog (13). We have found also that oral glucose ingestion suppresses plasma motilin in both normal subjects and diabetic patients (12). The present study was designed to investigate basal levels of motilin as well as the change after oral glucose loading in patients with hyperthyroidism and liver cirrhosis.

MATERIALS AND METHODS

Twelve normal subjects, 8 males and 4 females, aged 21–41, were studied. None of them had symptoms or signs of hepatic or gastrointestinal dysfunction or a family history of diabetes mellitus. They weighed less than 20% over the ideal body weight. Twelve patients with hyperthyroidism, 2 males and 10 females, aged 18–36, were studied. The diagnosis of hyperthyroidism was made on the basis of clinical symptoms and laboratory findings such as the T3 resin sponge uptake (42 to 58 %) and plasma thyroxine (16 to 34 μg/dl). Seven males and 3 females, aged 33–65, who were diagnosed as having liver cir-
rhosis based on histological findings of biopsied specimens of the liver, were also examined. After an overnight fasting, 50 g of glucose was given orally to all subjects early in the morning. Blood was withdrawn into heparinized disposable syringes from the antecubital vein immediately before and 30, 60, 90, 120, and 180 min after the glucose load. A 1 ml aliquot of the blood was used for the determination of blood glucose by the Technicon AutoAnalyzer (10). Another 2 ml aliquot of blood used for the determination of motilin was placed promptly into chilled tubes containing 2000 U of Trasylol in a volume of 0.2 ml. The mixture was immediately centrifuged at 4°C and plasma was separated and frozen at −20°C until assayed. Plasma motilin was measured by dextran-coated charcoal radioimmunoassay using antiseraum raised in a guinea pig against synthetic motilin. Synthetic motilin was used as standard and also for labeling. Labeled hormone was prepared by the chloramin T method (8). Motilin-free plasma prepared by the charcoal extraction procedure (1) was added to the standard. The minimal detectable quantity by this assay is 50 pg/ml. Intra- and inter-assay variations were less than 4.7% and 9.6%, respectively. The addition of synthetic motilin to normal plasma gave the mean (±SE) recovery rate of 98±12%. In our radioimmunoassay system, substance P (synthesized by Yanaihara), secretin (synthesized by Yanaihara), vasoactive intestinal polypeptide (synthesized by Yanaihara), human gastrin I (Imperial Chemical Industries, U.K.), somatostatin (synthesized by the Protein Research Foundation, Minoo, Osaka, Japan), porcine monocomponent glucagon (Novo, Denmark), and pork insulin (Novo, Denmark) did not cross react with motilin antiseraum. Serial dilution of plasma obtained from a normal subject, a hyperthyroid patient, and a patient with liver cirrhosis gave curves parallel to that of standard motilin. The motilin preparation used in the present experiments was synthesized by Yanaihara et al. and showed a very high purity, giving the expected amino acid composition after acid hydrolysis and behaving as a single component on thin-layer chromatography in two solvent systems. This synthetic polypeptide (21) enhanced motor activity of the stomach and duodenum in dogs during 30 min intravenous infusion at a dose of 5 ng/kg/min, similarly to the action of natural porcine motilin.

RESULTS

Effect of Oral Glucose Loading on Plasma Motilin in Normal Subjects

As shown in Fig. 1, the mean fasting plasma motilin in the 12 normal subjects was 224±36 pg/ml (±SE). Oral glucose loading gradually lowered plasma motilin levels to 126±26 pg/ml at 60 min, 106±17 pg/ml at 90 min, and 130±17 pg/ml at 120 min, which were significantly lower than the fasting values (P<0.05, P<0.01, and P<0.01, vs fasting level respectively, by paired Student 't' test). Plasma motilin increased thereafter, reaching the level of 276±47 pg/ml at 180 min, which was not significantly different from the fasting level. The mean blood glucose level rose from a fasting level of 80±3.2 mg/dl to a peak level of 139±9 mg/dl at 30 min after glucose administration.

Plasma Motilin Response to Oral Glucose Loading in Patients with Hyperthyroidism

The mean fasting plasma motilin level in the 12 hyperthyroid patients was 314±56 pg/ml, which was slightly higher but not significantly different from those in normal subjects. As shown in Fig. 1, plasma motilin levels at 60, 90, and 120 min after the glucose loading were 175±23 pg/ml, 171±30 pg/ml, and 188±26
pg/ml, respectively, which were either half or one third of fasting levels (P<0.02, P<0.01, P<0.001, respectively vs fasting level). In hyperthyroid patients, blood glucose levels after glucose loading were significantly higher than in normal subjects.

**Plasma Motilin Response to Oral Glucose Loading in Patients with Liver Cirrhosis**

In 10 patients with liver cirrhosis, fasting plasma motilin was 391 ± 71 pg/ml, which was much higher than in normal subjects (P<0.05). Following glucose administration plasma motilin was gradually lowered to the values of 200 ± 49 and 191 ± 59 pg/ml at 90 and 120 min, respectively, which were far below those in the fasting state (P<0.05, and P<0.05 respectively, vs fasting levels). Plasma motilin invariably tended to rise to the basal state thereafter. Fasting blood glucose levels in patients with liver cirrhosis were not significantly different from those in normal subjects. However, the blood glucose curve after glucose loading showed a mild diabetic pattern in most of the cases studied.

**DISCUSSION**

Several investigators have demonstrated that pharmacological actions of motilin are related to gastrointestinal motility (2, 3, 18), gastric secretion (2, 4, 18), pancreatic secretion (4, 14), and lower esophageal sphincter pressure (5, 7, 9). However, the exact physiological importance of this peptide still remains obscure. To solve this problem, the release of motilin under various physiological conditions must be clarified. Brown and Dryburgh (6) observed that duodenal alkalinization in dogs resulted in an increase of serum motilin, while Mitznegg et al. (16) observed that intraduodenal acidification in man enhances motilin release. These contradicting results still remain debatable, but are possibly due to the difference of species employed. We have previously found that plasma motilin fluctuates in the fasting state in man (12) and dog (13). The present study demonstrates that fasting plasma motilin levels were significantly higher in patients with liver cirrhosis but not in hyperthyroid patients than those in normal subjects. It was also observed that oral glucose administration lowered plasma motilin levels in patients with hyperthyroidism and liver cirrhosis. This finding regarding the suppressive effect of oral glucose administration on motilin release corresponds with the results obtained by Mitznegg et al. (17), who observed a tendency of decrease, though not significant, in plasma motilin after 130 g glucose loading. The mechanism by which glucose suppresses motilin release is still unknown. It may be either a direct effect of ingested glucose on motilin cells or via elevated glucose or insulin levels. Further studies on the mechanism of action of glucose are in progress in our laboratory. Although a rapid gastric emptying time (11) and elevated fasting plasma gastrin levels (20) have been demonstrated in hyperthyroidism, fasting plasma motilin levels and their response to the oral glucose loading were within normal limits in patients with hyperthyroidism. On the other hand, elevated fasting plasma motilin levels with the normal suppressive effect of glucose were observed in patients with liver cirrhosis. The reason for the elevation of fasting plasma motilin in patients with liver cirrhosis, though not clear at present, deserves consideration. Metabolic disorders or abnormal gastrointestinal functions in liver cirrhosis (15) may be responsible for this abnormality. Changed metabolism of motilin in the liver may also contribute to the elevation of plasma motilin especially in liver cirrhosis. Further studies should clarify the factors affecting plasma motilin levels not only in normal subjects but also in various diseased states.
REFERENCES


