Radioimmunoassay of Secretin
A Critical Review and Current Status

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The radioimmunoassay methods of secretin are reviewed with respect to production of antibody, preparation of radioactive tracers, and effect of plasma interference. The major difference in the secretin assay methods resides in handling plasma interference. Thus the assay sensitivity decreased markedly when the assay was conducted by diluting plasma samples. When the assay was conducted by compensating for plasma interference with homologous hormone-free plasma, the effect of plasma interference was greatly reduced, leading to a more sensitive assay. However, this method probably cannot obtain consistent results with plasma samples collected under various experimental conditions. The method is still subject to considerable desensitization and assay variation. On the other hand, the elimination of plasma interference before assay results in the most sensitive secretin assays capable of detecting consistently a significant postprandial rise in plasma secretin level. It is concluded that a sensitive, validated secretin radioimmunoassay should be one that is capable of detecting increments of plasma secretin in response to doses of intraduodenal acid at 0.055 mEq/min or lower and intravenous administration of exogenous secretin at 0.03 CU/kg/hr with concomitant stimulation of pancreatic bicarbonate and water secretion. With a sensitive and accurate radioimmunoassay for secretin, it is now possible to further investigate the physiology and pathophysiology of secretin.

In 1902, Bayliss and Starling (1) demonstrated that introduction of dilute hydrochloric acid into a vagally denervated loop of the jejunum in dog produced a marked pancreatic secretory response and that the same response could be produced by intravenous injection of a mucosal extract of the jejunal loop. They concluded that a stimulatory chemical substance was carried by the blood from the jejunum to the pancreas and named it "secretin." The term "hormone" was later designated to any substance, like secretin, produced by one organ that was carried by the blood to a target organ where its biological effect was manifested (2). Although secretin was the first gut hormone discovered, its purification from hog's small intestinal mucosa was achieved in 1961 by Jorpes and Mutt (3). Its chemical structure was soon determined as a heptacosapedptide of 3056 daltons (4). Subsequently, Bodanszky and coworkers (5, 6) successfully prepared synthetic secretin which was soon shown to have the same potency (7) and spectrum of known biological actions as the natural peptide (7, 8). The availability of large quantities of synthetic secretin prompted many workers to undertake extensive studies on the biological and physiological action of secretin during the past decade. Indeed, exogenous secretin, given intravenously, is the most potent stimulant of pancreatic secretion of water and bicarbonate. The production of pancreatic secretion rich in bicarbonate by the intraduodenal infusion of a dilute hydrochloric acid had been believed to result from release of endogenous secretin. However, the identity of the active factor discovered by Bayliss and Starling (1) was later shown to be secretin (2).
liss and Starling (1) as the polypeptide purified by Jorpes and Mutt (3) has not been established by a direct evidence. The attempts in the past decade to establish the hormonal status of secretin by the radioimmunoassay technique introduced by Berson and Yalow (9) had met with failure.

Previous studies by bioassay have indicated that the intraduodenal pH threshold for acid to stimulate pancreatic bicarbonate secretion in dogs was 4.0-4.5 (10, 11). The observation that pH of the first portion of duodenum in both humans (12, 13) and dogs (14) during the postprandial period was frequently at 4.5 or lower appeared to correlate well with the pH threshold of duodenal acidification in dogs. It was therefore reasonable to expect a significant rise in the circulating secretin level after ingestion of a meal. However, most of the investigators failed to detect a postprandial rise in the plasma secretin concentration by radioimmunoassay techniques that were consistently capable of detecting a significant rise in the blood following duodenal acidification (15-22). The hormonal status of secretin was therefore questioned by some investigators (23-25). In recent years, using modified radioimmunoassay methods, a small but significant postprandial increase in plasma immunoreactive secretin has been observed in both humans (13, 26-28) and dogs (29). These observations together with our most recent observation in dogs, that intravenous administration of a specific rabbit antisecretin serum produced a profound inhibition on endogenous and exogenous secretin-stimulated pancreatic secretion of water and bicarbonate (30), thus appear to support the original postulate of Bayliss and Starling (1) that secretin is a hormone.

In the present communication we wish to discuss the problems encountered in the radioimmunoassay of secretin and compare the techniques used by various laboratories so as to analyze the importance of the results of various studies on plasma secretin concentration in relation to the physiological function of this hormone.

RADIOIMMUNOASSAY METHOD

Production of Antibody

Antibody against porcine secretin can be produced in rabbits by immunization with either conjugated or unconjugated secretin, as reported by Boehm et al (31) and Fahrenkrug et al (32). The chance of obtaining a high-titer antiserum, however, is better when a conjugated secretin is used for immunization (31, 32). Experimentally, the titer of an antiserum is determined by measuring the binding of a fixed amount of tracer with various dilutions of antiserum and expressed as the dilution at which 50% of tracer is bound. The final dilutions of various antisecretin sera reported in the literature are summarized in Table 1. Most of the antisera produced by conjugated secretin, generally to bovine serum albumin (BSA), had titers above 1 : 10^5, whereas greater variation appeared when unconjugated secretin was used for immunization. The results of Straus et al (33) indicated that the guinea pig is probably not a good species to produce antisecretin serum. It should be noted that the titer of an antiserum depends on many variables, including specific radioactivity and immunoreactivity of the tracer, concentration and avidity of the specific immunoglobulins, and assay conditions. For example, the titer of the antiserum used by Boden and Chey (35) was 1 : 75,000 [1 : 150,000 by Boden (36)] which was increased to 1 : 300,000 through the use of a preparation of [125I]secretin with higher specific activity (37). The titers of the antisera used by Rayford et al (46) and Raptis et al (40) could have been higher since they used the low immunoreactive [125I]6-tyrosylsecretin (33, 48, 49) as a tracer. Furthermore, the titer of a given antiserum measured after the tracer and the antibody have reached equilibrium would be higher than that measured before equilibrium is established. The latter condition is often used in the actual radioimmunoassay. Therefore, the titer alone cannot adequately describe the property of a given antibody and further characterization is required.

Characterization of Antibody

In addition to the titer, the antibody also should be characterized with respect to specificity and affinity for the antigen as well as its own heterogeneity.

Specificity. Most investigators reported on antisecretin sera exhibiting no cross-reactivity toward other polypeptide hormones, namely, insulin, glucagon, cholecystokinin (CCK), gastrin I, motilin, and gastric inhibitory polypeptide (GIP) at concentrations as high as 10^{-9} M. A slight cross-reaction by vasoactive intestinal polypeptide (VIP), generally less than 0.5% compared with secretin, was occasionally reported (17, 30-32, 39, 42, 46, 50). Since saturation of secretin binding was achieved at concentrations below 5 \times 10^{-9} M, cross-reactivity of VIP generally would not be a

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