RADIOIMMUNOASSAY OF SECRETIN IN HUMAN SERUM

J. D. Young, L. Lazarus and D. J. Chisholm

Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, Australia

and F. F. V. Atkinson

National Biological Standards Laboratory, Canberra A.C.T., Australia

It has been suggested by Elrick (*1*) and Dupre (*2*) that a gastrointestinal hormone is responsible for the increase in insulin release following an oral glucose load and that secretin is the hormone. Proof of this assumption requires a method for measuring secretin in blood. We wish to describe here a radioimmunoassay system for measuring secretin in serum using ^{125}I-labeled synthetic secretin, antiserum prepared (in rabbits) to porcine secretin and an ion-exchange resin to separate the "free" labeled hormone.

MATERIALS AND METHOD

Synthetic secretin was obtained as gifts from J. E. Jorpes and M. A. Ondetti (see acknowledgments). Because of the scarcity of the material, it was used only for the preparation of the labeled antigen. Because porcine secretin (Sigma Chemical Co., Batch 126B-1720) was used as the antigen standard, the results are expressed in millimicrograms of the Sigma preparation which had an immunological potency of approximately 1/100 the potency of the synthetic material and a stated biological potency of 40 Crick units/mg. It was found that solutions of the antigen standard would keep up to 8 weeks when stored at −20°C.

All dilutions were made with phosphate buffer (pH 7.3, 0.15 *M*) to which was added recrystallized bovine serum albumin to a concentration of 0.5% W/V. To simulate the nonspecific protein binding of human serum, the standards (0–20 mμg) were set up in 0.5 ml of 3.75% W/V gelatine solution (*3*).

Antiserum to porcine secretin was prepared by intramuscular injections twice weekly in Freud's incomplete adjuvant of 25 units of porcine secretin conjugated to rabbit serum albumin (*4*). The ideal dilution of antiserum was found to be 1/500.

^{125}I-secretin was prepared by the method of Hunter and Greenwood (*5*), the only modification being that the pH of the secretin solution was raised to 8.7 It was possible to achieve a specific activity of about 75–100 mCi/mg. This comparatively low specific activity may be due to the lack of tyrosine residues in secretin (*6*) and the need to iodinate other amino acid residues such as histidine (*7*).

It was found that 5-ml aliquots containing about 10 mμg of the labeled hormone could be stored at −20°C for periods of 2–3 weeks. After this time there was a significant decrease in immunological potency, and the labeled material was discarded.

Amberlite CG 400 type 2 hydroxide cycle anion exchange resin was prepared by the method of Lazarus and Young (*8*). The method of assay consisted of mixing in a scintillation vial 0.5 ml of serum for assay or the 3.75% gelatine solution containing appropriate standard, 0.05 mμg of ^{125}I-secretin, and 0.5 ml of the diluted antiserum. The reactants were then diluted to 5 ml with phosphate buffer and incubated at 37°C for 14 hr. Two hundred milligrams of ion-exchange resin was added to the mixture, and the vial was then shaken in a fast-swing arm shaker at 180 oscillations/min at 20°C for 2 hr. The resin which takes up the free hormone was washed with 50 ml of distilled water, and the radioactivity bound to the resin was measured in a well gamma scintillation counter and expressed as a percentage of the added tracer. From the percent resin uptake of the standards the concentration of hormone in the serum can be determined graphically.

DISCUSSION

The assay was specific for secretin because no cross reaction was observed with insulin, glucagon, gastrin or highly purified pancreozymin-CCK in the assay

Received July 8, 1968; revision accepted Sept. 3, 1968.

For reprints contact: J. D. Young, St. Vincent's Hospital, Garvan Institute of Medical Research, Darlinghurst 2010, N.S.W., Australia.

system. Serial dilutions of a human serum sample with an elevated secretin level gave a dose response that was parallel to the response obtained with both the Sigma porcine and the highly purified Jorpes porcine preparations (Fig. 1).

The assay was sensitive; i.e., it was capable of measuring 0.4 mμg with duplicates agreeing at this level to ± 10%. The effect of incubation at various temperatures was studied in an attempt to demonstrate secretinase activity (*9*). Preincubation of serum at various temperatures up to 56°C for 2 hr failed to show loss of secretin activity in a number of serum samples.

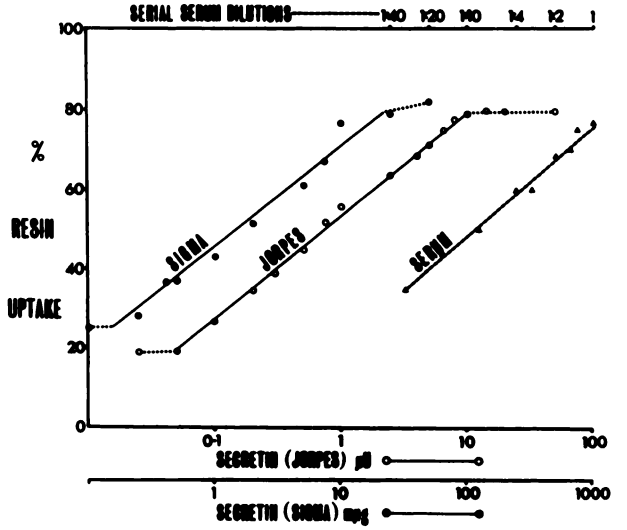

FIG. 1. Standard log dose response of Sigma porcine secretin standard (40 units/mg), highly purified porcine secretion (J. E. Jorpes 3,000–5,000 units/mg) and a serum sample serially diluted with 3.75% gelatine.

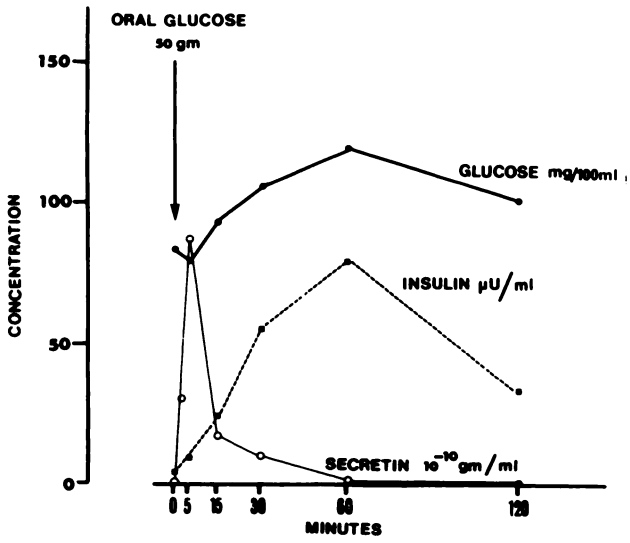

FIG. 2. Blood glucose, serum insulin and serum secretin responses to an oral glucose load.

The serum secretin level in 13 healthy subjects after a 12-hr fast was less than 0.4 mμg/ml. Studies were performed of the serum secretin levels during a 50-gm oral glucose tolerance test in six of the above healthy subjects. The results revealed that there was an elevation of the serum secretin to a mean of 15 mμg/ml (range 7.5–25) occurring shortly (2–10 min) after ingestion of the glucose and well before the peak of serum insulin (Fig. 2).

These results demonstrate that secretin is released after the oral administration of glucose, and they support the hypothesis that secretin is responsible for the augmentation of insulin release after an oral glucose load.

SUMMARY

A radioimmunoassay technique has been set up to measure secretin in human serum. It has been shown that a pulse of immunoreactive secretin is released shortly (5 min) after an oral glucose load.

ACKNOWLEDGMENTS

We acknowledge with thanks the generous gifts of synthetic secretin from both M. A. Ondetti, The Squibb Institute for Medical Research, New Brunswick, N.J. and J. E. Jorpes, Chemistry Dept., 11 Karolinska Institutet, Stockholm, Sweden.

REFERENCES

1. ELRICK, H., STIMMLER, L., HLAD, C. J. AND ARAI, Y.: Plasma insulin response to oral and intravenous glucose. *J. Clin. Endocrinol. Metab.* **24**:1,076, 1964.

2. DUPRE, J.: An intestinal hormone affecting glucose disposal in man. *Lancet* **2**:673, 1964.

3. YOUNG, J. D. AND KRAEGEN, E. W.: Simultaneous assay of insulin and glucagon in serum. *Australian J. Exp. Biol. Med. Sci.* In press.

4. GOODFRIEND, T. L., LEVINE, L. AND FASMAN, G. D.: Antibodies to bradykinin and angiotensin: a use of carbodiimides in immunology. *Science* **144**:1,344, 1964.

5. HUNTER, W. M. AND GREENWOOD, F. C.: Preparation of iodine-I-131 labelled growth hormone of high specific activity. *Nature* **194**:495, 1962.

6. MUTT, V. AND JORPES, J. E.: Contemporary developments in the bio-chemistry of the gastrointestinal hormones. *Recent Progr. Hormone Res.* **23**:483, 1967.

7. IZZO, J. L., RONCONE, A., IZZO, M. J. AND BALE, W. F.: Relationship between degree of iodination of insulin and its biological electrophoretic and immunochemical properties. *J. Biol. Chem.* **239**:3,749, 1964.

8. LAZARUS, L. AND YOUNG, J. D.: Radioimmunoassay of human growth hormone using ion exchange resin. *J. Clin. Endocrinol. Metab.* **26**:213, 1966.

9. GREENGARD, H., STEIN, J. F. AND IVY, A. C.: *Am. J. Physiol.* **133**:121, 1941.