REVISTA ESPAÑOLA DE FISIOLOGIA, 36, 57-62. 1980

«Big Big» Gastrin Release by the Isolated Islets of Langerhans Incubated *in vitro*

P. Rotllan, O. García-Hermida and J. Gómez-Acebo

Instituto «Gregorio Marañón»
Velázquez, 144. Madrid-6 (Spain)

(Received on May 14, 1979)

P. ROTLLAN, O. GARCIA-HERMIDA and J. GOMEZ-ACEBO. *«Big Big» Gastrin Release by the Isolated Islets of Langerhans Incubated* in vitro. Rev. esp. Fisiol., 36, 57-62. 1980.

The immunoreactive form of gastrin released by the islets and some of the characteristics of this release have been studied. This gastrin released by the islets in the present experiments corresponds to what has been named «Big Big» gastrin in serum of patients with the Zollinger-Ellison syndrome, in normal human serum and in extracts of proximal jejunum. Most of the «Big Big» gastrin released from the islets corresponds to spontaneous release.

Whether normal islet tissue produces gastrin has been a matter of controversy. The presence of gastrin in normal pancreatic islets by means of immunofluorescence has been demonstrated (7, 8, 13, 19). Moreover by employing a sensitive bioassay, pancreatic extracts from normal dogs and rabbits have now been found to contain an acid stimulating substance different from histamine (16). Recently several investigators have reported immunoassayable gastrin in extracts of normal mammalian pancreatic tissue (2, 7, 14, 15, 18, 20, 21, 23, 24). So far, however, few studies have been reported on secretion of gastrin from normal pancreas and of cultured islets (3, 20, 21). Considering that up to the present there is no knowledge available on the characteristics of the gastrin released by the islets of Langerhans in an incubation system, we think our study is important since, there are at present six chemically defined molecular forms of gastrin with known biological activity and three other immunoreactive forms with uncertain structure and unknown biological activity (25).

Materials and Methods

Islets were isolated by the collagenase technique (10, 11) from the pancreas of fed male Wistar rats of 300-350 g. Batches of 50 islets were incubated at 37° C for 90 minutes in 700 μl of a bicarbonate buffer medium (22) containing albumin (0.5 % w/v, bovine plasma albumin fraction V, Sigma); glucose (D+-glucose, Merck); and as required L-alanine; L-arginine; glycocoll (Sigma Chemical Co.); Octanoate (Fluka AG, Buchs); Tolbuta-

mide (Hoechst Ibérica, Barcelona). At the end of the incubation period the media were kept for the assay of gastrin. In another set of experiments, batches of 100 islets were preincubated for 30 minutes in 1.4 ml of the medium previously described. After this 30-minute period the incubation medium was changed, islets then were placed in 1.4 ml of fresh medium and stimulated with glucose in concentrations of 50, 150 and 300 mg/ml. Samples were taken after 30, 60 and 90 minutes and IRGa was determined in these and in the preincubation medium. At least 6 series of experiments were performed for each experimental condition, in each series controls and problems were done with the islets from a single pancreas.

Gastrin release into the medium was determined by radioimmunoassay (C.I.S.) using synthetic human gastrin I as standard. Antibodies were raised in rabbits by immunization with synthetic human gastrin I conjugated to bovine serum albumin. The gastrin labelled with ^{125}I, was synthetic human gastrin I. Bound and free hormones were separated with dextran coated charcoal. The sensitivity of the assay is 2 pg/ml. The IRGa released is expressed as pg/50 islets/90 minutes. The medium proceeding from the incubations was dialyzed, lyophilized and stored at —20° C until fractionation.

Columns of Sephadex G-50 fine, 1 ×50 cm were equilibrated with barbital buffer 0.02 M, pH 8.6 containing 0.25 % serum albumin. Samples were reconstituted, fortified with ^{125}I-albumin, ^{125}I-insulin and Na ^{125}I and applied to the columns and eluted with the same albumin-barbital buffer used to equilibrate the columns. The zone of emergence of the component was plotted as «percent of elution volume» between protein peak (0 %) and salt peak (100 %). Flow rates were approximately 10 ml per hour and 1 ml fractions were collected for assay. Gastrin concentrations in the various fractions were determined by the method of radioimmunoassay previously described. Eluates containing the immunoreactive fraction were combined and fractionated again on Sephadex G-50 columns.

Results

IRGa could be measured in the incubation medium of isolated islets. In a glucose-free buffer the concentration of IRGa was 110 ± pg/ml/50 islets/90 minutes. Variation of glucose concentration in the incubation medium was without effect on the IRGa release (table I). The effect and respective concentrations used of L-alanine, L-arginine, glycocoll, octanoate and tolbutamide on the release of IRGa from isolated rat islets are shown in table II.

Table I. *Concentration of immunoreactive gastrin (IRGa) (mean ± SD in pg/ml) in the incubation medium in response to different concentrations of glucose.*
( ) Number of experiments.

Glucose mg/100 ml	IRGa pg/ml/50 islets 90 min incubation
—	110 ± 16 (15)
50	108 ± 11 (52)
150	115 ± 12 (52)
300	104 ± 10 (15)

Table II. *Concentration of immunoreactive gastrin (IRGa) (mean ± SD pg/ml) in the incubation medium during stimulation with L-arginine, L-alanine, glycocoll, octanoate and tolbutamide.*
Number of experiments per group, 12.

Stimulus	IRGa pg/ml/50 islets 90 min incubation
L-arginine (10 mM)	112 ± 16
L-alanine (10 mM)	94 ± 12
Glycocoll (10 mM)	101 ± 17
Octanoate (10 mM)	113 ± 12
Tolbutamide (0.3 mg/ml)	116 ± 18

None of the stimulus used, at least in the dose used in the present study, had a clear effect on pancreatic gastrin release.

Dynamics of IRGa release. After preincubation for 30 minutes with buffer glucose-free, the islets were exposed to increasing concentrations of glucose (50, 150 and 300 mg/100 ml) for 90 minutes (fig. 1). The highest IRGa output corresponds to the preincubation period, the mean value of IRGa secreted during this period was 79 ± 13 pg/ml/50 islets, which represents approximately 70 % of the total IRGa secreted during the whole incubation period.

IRGa components released from the isolated islets. On gel filtration, the only immunoreactive component present in the medium, resulting from the incubation of the isolated islets, was found in the eluates emerging from the Sephadex filtration in the albumin region (fig. 2A) corresponding to what has been called «Big Big» gastrin (26). On refractionation, the «Big Big» peak was eluted in the same region, as illustrated in figure 2B.

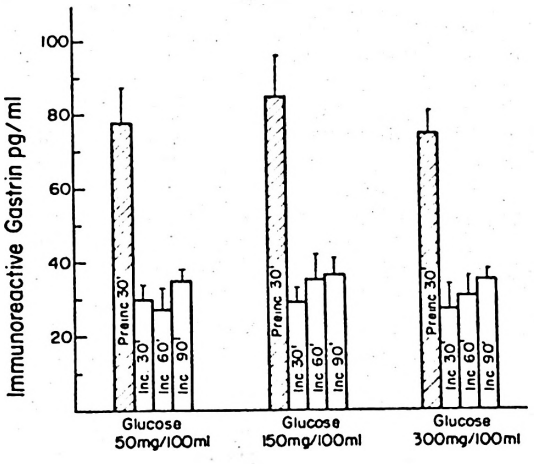

Fig. 1. *Effect of different concentrations of glucose on the immunoreactive gastrin (IRGa) content of incubation medium during a 30-minute preincubation period and after 30, 60 and 90 minutes of incubation.*
IRGa is expressed as pg/ml/50 islets. The results are means of at least 12 experiments ± SD.

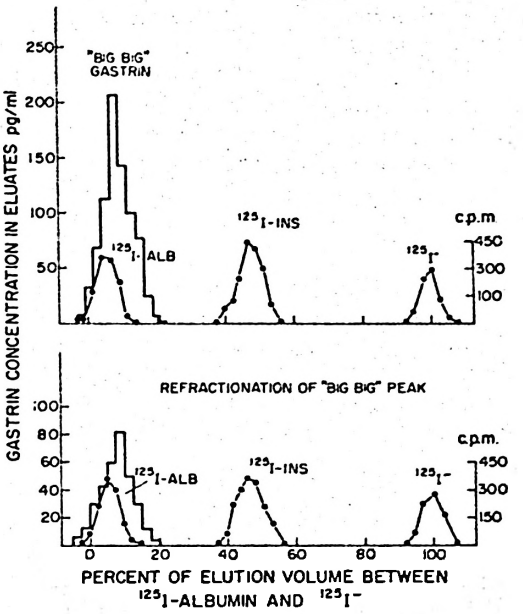

Fig. 2. *Distribution of immunoreactive gastrin on Sephadex G-50 gel filtration in sample proceeding from the incubation mediums.*
The concentrations of labeled marker molecules are shown as solid circles. (Bottom) refractionation of «Big Big» peak.

Discussion

Our findings indicate that immunoreactive gastrin released by the isolated rat islets of Langerhans corresponds to what has been called «Big Big» gastrin, as demonstrated by gel filtration to be present in plasma from patients with the Zollinger-Ellison syndrome and in extracts of proximal jejunum (26). This form of immunoreactive gastrin has been demonstrated to be a major fraction of total gastrin immunoreactivity in normal human, canine and porcine plasmas in the nonstimulated state and is the only form

detectable in gastrectomized patients post Billroth II (27).

Considering that the most abundant form of gastrin in gastric tissue extracts is G-17, accounting for 90 to 95 % of extractable gastrin, the rest being G-34 (25), our findings could suggest that most of the «Big Big» gastrin found in the normal plasma most probably has its origin in this gastrin component released by the islets of Langerhans. At present four different type of cells are known to be the cellular components of the islets and five hormones are present in these cells. The B-cells produce insulin (9), A-cells produce glucagon (1), the enterochromaffin cell produces PP (12) and the D-cells that contain somatostatin (4, 5). Most likely D-cells may also release «Big Big» gastrin. The presence of gastrin has been demonstrated by immunofluorescence in these cells (6, 13) which also contain two different types of secretory granules (17). Recently it has also been shown that cultured islets of Langerhans are capable of releasing pancreatic gastrin after stimulation with cyclic AMP and aminophilin (3).

Our results indicate that, in the experimental model used, most of the «Big Big» gastrin released corresponds to «spontaneous» release and none of the substances used could be considered as a stimulant for this release. The difficulty in detecting variations of IRGa in the medium proceeding from the 90 minutes incubation period, could be due to the fact that the exact nature of the IRGa in the normal islets is still unknown. If its structure differs significantly from that of antral gastrin, with a subsequent difference in binding sites for the antibodies, raised against heptadecapeptide gastrin, it is possible that normal islets secrete the gastrin immunoreactive component in greater amounts when expressed in absolute concentrations, i.e., when antibodies against «Big Big» gastrin or pancreatic gastrin are obtained.

Acknowledgments

This work has been supported by a research grant (613/01) from «Comisión Asesora para el Desarrollo de la Investigación».

Resumen

Se estudian las distintas formas de gastrina inmunorreactiva liberadas por islotes aislados de rata y alguna de las características de este proceso de liberación. La gastrina segregada por los islotes aislados de rata se corresponde con lo que se ha convenido en llamar «Big Big» gastrina, presente en el suero de pacientes con el síndrome de Zollinger-Ellison, en suero humano y en extractos de la parte proximal del yeyuno. La mayor parte de la «Big Big» gastrina se libera de forma espontánea.

References

1. BAUM, J., SIMONS, B. E. (Jr), UNGER, R. H. and MADISON, L. L.: *Diabetes*, **2**, 371-374, 1962.
2. BECKER, H. D., REEDER, D. D. and THOMPSON, J. C.: *Surgery*, **74**, 778-781, 1973.
3. BRAATEN, J. T., GREIDER, M. H., MCGUIGAN, J. E. and MINTZ, D. H.: *Endocrinology*, **99**, 684-691, 1976.
4. ERLANDSEN, S. L., HEGRE, O. D., PARSONS, J. A., MCEVOY, R. C. and ELDE, R. P.: *J. Histochem. Cytochem.*, **24**, 883-897, 1976.
5. GOLDSMITH, P. C., ROSE, J. C., ARIMURA, A. and GANONG, W. F.: *Endocrinology*, **97**, 1061-1064, 1975.
6. GREIDER, M. H. and MCGUIGAN, J. E.: *Amer. J. Pathol.*, **59**, 76a-77a, 1970.
7. GREIDER, M. H. and MCGUIGAN, J. E.: *Diabetes*, **20**, 389-396, 1971.
8. HANSKY, J., MCNAUGHTAN, J. and NAIRN, R. C.: *Aust. J. Exp. Biol. Med. Sci.*, **50**, 391-396, 1972.
9. HARTROFT, W. S. and WRENSHALL, G. A.: *Diabetes*, **4**, 1-7, 1955.
10. LACY, P. E., YOUNG, D. Z. and FINK, C. J.: *Endocrinology*, **83**, 1155-1161, 1968.
12. LARSSON, L. I., SUNDLER, F. and HOKANSON, R.: *Diabetologia*, **12**, 211-226, 1976.
13. LOMSKY, R., LANGR, F. and VORTEL, V.: *Nature* (Lond.), **223**, 618-619, 1969.
14. MCGUIGAN, J. E.: *Gastroenterology*, **55**, 315-327, 1968.

15. NILSSON, G., YALOW, R. S. and BERSON, S. A.: In «Frontiers in Gastrointestinal Hormone Research» (S. Anderson, ed.). Almquist and Wiksell, Stockholm, 1973, pp. 95-101.
16. OMOLE, A. A., AMURI, B. O., EXIMOKHAI, M. and AYANDIPO, A.: *Nature New Biol. (London)*, **237**, 183-184, 1977.
17. PARRILLA, R., GARGÍA HERMIDA, O. and GÓMEZ-ACEBO, J.: *Acta Diabetol. Lat.*, **10**, 819-840, 1973.
18. POINTER, H., ACCARY, J. P., VATIER, J., DUBRASQUET, M. and BONFILS, S.: *Horm. Metab. Res.*, **5**, 303-304, 1973.
19. POLAK, J. M., STAGG, B. and PEARSE, A. G. E.: *Gut*, **13**, 501-512, 1972.
20. REHFELD, J. F. and IVERSEN, J.: *Excerpta Medica International Congress Series*, **280**, 52, 1973.
21. REHFELD, J. F. and IVERSEN, J.: *Horm. Metab. Res.*, **6**, 260-264, 1974.
22. RENOLD, A. E., MARTIN, D. B., DAGENAIS, Y. M., STEINKE, J., NICKERSON, R. and SHEPS, M. C.: *J. Clin. Invest.*, **39**, 1487-1493, 1960.
23. STREMPLE, J. F.: *Surg. Clin. North Am.*, **55**, 303-310, 1975.
24. THOMPSON, J. C., REEDER, D. D., DAVIDSON, W. D., JACKSON, B. M. and CLENDINNEN, B. G.: In «Frontiers in Gastrointestinal Hormone Research» (S. Anderson, ed.). Almquist and Wiksell, Stockholm, 1973, pp. 111-133.
25. WALHS, J. H.: *Fed. Proc.*, **36**, 1948-1951, 1977.
26. YALOW, R S. and BERSON, S. A.: *Biochem. Biophys. Res. Commun.*, **48**, 391-394, 1972.
27. YALOW, R. S. and WU, N.: *Gastroenterology*, **65**, 19-27, 1973.