Diabetologia 5, 349–352 (1969)

SHORT COMMUNICATIONS

Chronic Effects of Insulin and Glucagon Upon Islet Function

W. Malaisse and F. Malaisse-Lagae

Laboratories of Experimental Medicine and Anatomo-Pathology, Brussels University, Brussels, Belgium

Received: November 22, 1968

Summary. Chronic administration of insulin to normal rats provokes a progressive reduction in both the pancreatic insulin content and the secretory responsiveness to glucose. The reduction of the secretory capacity is more marked than that of the stores of hormone. These results suggest that hyperinsulinism inhibits the secretory process within the beta-cell independently of its effects upon insulin synthesis. Prolonged treatment with zinc-glucagon also causes a reduction of the amount of insulin stored and secreted by the pancreas, but the ratio of insulin output to content is not significantly modified. The chronic influence of hyperglucagonism could thus correspond to a net catabolic effect upon the beta-cell. It is suggested that the reduced insulin-secretory capacity observed in experimental hyperinsulinism and hyperglucagonism could play a role in the impairment of glucose tolerance seen in the corresponding clinical conditions.

Influences chroniques de l'insuline et du glucagon sur la fonction insulaire

Résumé. L'administration chronique d'insuline à des rats normaux provoque un abaissement progressif des réserves pancréatiques d'insuline et de la riposte sécrétoire du pancréas au glucose. La réduction de la capacité sécrétoire est plus marquée que celle des réserves hormonales. Ces données suggèrent que l'hyperinsulinisme inhibe l'activité sécrétoire des cellules beta indépendamment de ses effets sur la biosynthèse d'insuline. L'administration prolongée de zinc-glucagon entraîne également une réduction des réserves pancréatiques d'insuline et de la capacité insulino-sécrétoire; le rapport entre le débit et le contenu insuliniques n'est cependant pas significativement modifié. Les modifications de la fonction insulaire au cours de l'hyperglucagonisme chronique semblent donc correspondre à un effet net catabolique sur les cellules beta. La réduction de la capacité insulino-sécrétoire au cours de l'hyperinsulinisme et de l'hyperglucagonisme expérimentaux pourrait expliquer l'intolérance glucidique qui caractérise les états cliniques correspondants.

Chronische Effekte von Insulin und Glucagon auf die Inselfunktion

Zusammenfassung. Chronische Verabreichung von Insulin an normale Ratten bewirkt eine fortschreitende Verringerung des Insulingehaltes und der Stimulierbarkeit des Inselzellsystems durch Glucose. Die Einschränkung der Sekretionskapazität ist dabei ausgeprägter als die der Hormonreserven. Die Ergebnisse deuten darauf hin, daß der Hyperinsulinismus den Sekretionsprozeß in der B-Zelle unabhängig von seinen Effekten auf die Insulinsynthese beeinträchtigt. Längere Behandlung mit Zink-Glucagon verringert ebenfalls die Menge des vom Pankreas gespeicherten und sezernierten Insulins, verändert aber das Verhältnis von Insulinausschüttung zum Insulingehalt nicht signifikant. Die chronischen Auswirkungen des Hyperglucagonismus könnten also einen rein katabolen Effekt auf die B-Zelle darstellen. Es ist anzunehmen, daß die Einschränkung der Sekretionskapazität für Insulin, die beim experimentellen Hyperinsulinsmus und Hyperglucagonismus beobachtet wird, im Zusammenhang mit den Störungen der Glucostoleranz steht, wie sie unter den entsprechenden klinischen Bedingungen auftreten.

Key-words: Hyperinsulinism, hyperglucagonism insulin secretion.

The insulin secretory function of the beta-cell is influenced by a series of hormones. Epinephrine and other adrenergic agents inhibit, whereas glucagon and some gastro-intestinal hormones enhance the stimulant action of glucose upon insulin secretion. These hormonal effects are both immediate and direct, and can be demonstrated by addition of the hormones to incubation media containing pancreatic tissue or isolated islets of Langerhans [15, 10, 8]. Other hormones including growth hormone [16], cortisol [12] and thyroxine [11] do not exert such immediate and direct effects. However, when administered *in vivo* for 2 to 5 days, these hormones cause marked alterations in the secretory responsiveness of the beta-cell to glucose [16, 12, 11]. The aim of the present investigation is to extend the study of the chronic hormonal influence upon islet function to the pancreatic hormones, insulin and glucagon.

Material and Methods

Rats. Two groups of female albino rats (Wistar; Rega, Heverlee) were examined. In the first group, the animals received each day at 6 p.m. a subcutaneous injection of insulin (4 U/rat; 33% Novo Actrapid Insulin and 67% Novo Lente Insulin; Novo Industri, Copenhagen). These rats were killed 40 or 88 h after the first injection of insulin. In the second group, rats were in-

jected subcutaneously twice daily with 0.1 mg zinc-glucagon (Zn-glucagon; Novo Research Institute, Copenhagen) for 4 days prior to killing. All animals had free access to food (Aliments Protector, Brussels) and water until killed by decapitation. Each treated rat was examined together with a control animal of same age and sex.

Experimental procedure. After decapitation, blood was collected and the plasma separated for sugar estimation with the Technicon Auto Analyzer using a method based on that of HOFFMAN [3]. The pancreas was removed and divided into small pieces (*ca.* 8 mg each). These were placed in groups of four into bicarbonate-buffered media (2 ml) containing glucose (150 mg/100 ml), bovine serum albumin (0.5%, w/v; bovine albumin, Fraction V; Sigma Chemical Co., St. Louis) and guinea-pig anti-insulin serum (lot 404, kindly donated by Dr. P.H. WRIGHT, Indiana University, Indianapolis). Sufficient anti-insulin serum was added to bind about twice the expected amount of secreted hormone, insulin secretion over 90 min of incubation at 36°C being equated to the fall in insulin-binding capacity of the medium. After incubation, all pieces of pancreatic tissue from the same animal were homogenized and extracted with acid-alcohol. The methods for assay of insulin secretion *in vitro* and the insulin content of the pancreas are described in detail elsewhere [13, 14].

Results

Administration of insulin for 2 to 4 days failed to modify significantly the body weight, the weight of the pancreas, and the plasma sugar concentration found at the time of killing, 16 h after the last injection of insulin. After 2 days of treatment with insulin, there was a minor but significant decrease in the pancreatic stores of insulin (−11 per cent), and a more marked decrease in the secretory response of the pancreas to glucose (−30 per cent). After 4 days of insulin administration, these changes in islet function were accentuated: the pancreatic stores of insulin were reduced by one-third and the rate of secretion evoked by glucose by two-thirds. The ratio of insulin output to content was thus markedly reduced.

The rats used as controls for comparison with those receiving Zn-glucagon were comparable with the control rats in the first group. They gained weight at a steady rate (+0.9±0.2 g/day). By contrast, the animals treated with Zn-glucagon lost 20 g over the 4 days-period of treatment. The weight of the pancreas was also reduced (−32 per cent). The insulin content and secretory capacity of the pancreas appeared normal when related to the weight of tissue, but were significantly reduced when calculated for the pancreas as a whole. The ratio of insulin output to content, however, was not significantly modified.

Table. *Chronic effects of insulin and glucagon upon islet function*

Mean values (± SEM) for body weight (4 days prior to and at death), plasma sugar concentration, pancreas weight, and insulin content and output are shown together with the statistical significance (a : $P < 0.02$; b : $P < 0.01$; c : $P < 0.001$) of differences between mean values for treated rats and their respective controls. The number of observations (in parentheses) refers to the number of animals examined in each group, except where insulin output is related to the weight of incubated tissue (μU/mg tissue). Such secretion was calculated from observations made in individual flasks, enough pancreatic tissue being obtained from each animal to be distributed into 9 flasks

Rats	Normal		Insulin 4 U/d × 2 d		Insulin 4 U/d × 4 d		Normal		Zn-Glucagon 0.2 mg/d × 4 d	
Body weight (g)										
— 4 days prior to death	167±3	(16)	165±4	(8)	166±5	(7)	160±3	(8)	161±3	(8)
— at death	171±3	(16)	169±4	(8)	170±5	(7)	163±3	(8)	141±2[c]	(8)
Plasma sugar (mg/100 ml)	135±2	(16)	129±2	(8)	131±11	(7)	137±3	(8)	135±3	(8)
Pancreas weight (mg)	617±16	(16)	609±17	(8)	623±23	(7)	622±16	(8)	425±34[c]	(8)
Insulin content										
— mU/mg tissue	3.01±0.12	(16)	2.75±0.11[b]	(8)	1.89±0.13[b]	(7)	2.93±0.21	(8)	2.90±0.49	(8)
— U/pancreas	1.85±0.08	(16)	1.67±0.05[a]	(8)	1.17±0.11[a]	(7)	1.81±0.10	(8)	1.13±0.11[c]	(8)
Insulin output (per 90 min)										
— μU/mg tissue	31.5±1.2	(144)	23.1±1.6[c]	(72)	9.6±1.2[c]	(63)	35.4±1.9	(72)	27.5±3.4	(72)
—mU/pancreas	19.8±1.0	(16)	14.1±1.6[b]	(8)	6.1±1.5[c]	(7)	21.9±1.3	(8)	10.1±1.7[c]	(8)
— μU/mU stored	10.9±0.8	(16)	8.6±1.1	(8)	5.1±1.1[b]	(7)	12.2±0.9	(8)	9.6±1.8	(8)

Discussion

The findings in insulin-treated rats are in agreement with the reduction in pancreatic insulin content found by HAIST [2] in rats treated for 7 days with exogenous insulin, and the reduced secretory response to glucose observed by Sodoyez and his colleagues (personal communication) on incubation of non-tumoural pancreatic tissue removed from golden hamsters bearing an insulin-producing tumour. These changes in islet function during experimental hyperinsulinism re-

semble those observed during fasting [14]. Thus, in both cases, the pancreatic insulin content and the secretory response to glucose are decreased. In both cases also, the reduction of the secretory activity is much more marked than that of the pancreatic stores of hormone. This phenomenon is illustrated by the progressive fall in the ratio of insulin output to content. After 4 days of treatment with insulin, this ratio is below half its control value, suggesting that insulin administration inhibits the secretory process within the beta-cell independently of its inhibitory effect upon insulin synthesis.

Administration of Zn-glucagon like that of insulin, reduced the total amount of insulin stored in the pancreas and the rate of secretion evoked by glucose *in vitro*. However, in the glucagon-treated rats, in contrast to the insulin-treated rats, the ratio of insulin output to content was not significantly modified, even after 4 days of treatment. Thus, relative to the amount of stored insulin, responsiveness to glucose appeared unaffected by induction of hyperglucagonism. In addition to the changes in islet function, Zn-glucagon administration also provoked concomitant losses in body and pancreas weights. The picture seen in these rats is almost the same as that observed after administration for the same period of large doses of thyroxine to normal rats [11]. This similarity suggests that the chronic action of Zn-glucagon upon islet function, like that of thyroxine, is mainly due to an overall catabolic effect upon various tissues including the islet beta-cells. Impairment of islet function under these experimental conditions is also compatible with progressive failure of the beta-cell, leading to meta-glucagon [6] or meta-thyroid [4] diabetes.

Whether the chronic effects of hyperinsulinism and hyperglucagonism upon islet function are due to a direct or indirect mechanism is unknown. Hyperinsulinism, as well as fasting, could influence islet function indirectly by lowering the plasma sugar concentration. It should be noted, however, that the insulin-treated rats had free access to food throughout the period of treatment, and were not hypoglycaemic at the time of death. Moreover, during fasting, there is no parallelism between the modifications of the secretory ability of the pancreas and the plasma sugar concentration: when the latter rises back to normal values after an initial fall, the secretory responsiveness of the pancreas remains depressed [14]. Finally, an abnormally low plasma sugar concentration is sometimes associated with an increased secretory activity of the beta-cell, for instance in late pregnancy [9] and in infection with trypanosomes [7]. One should therefore not exclude the hypothesis of a direct but delayed effect of hyperinsulinism upon the beta-cell. A similar delayed but direct effect may occur in hyperglucagonism, independently of the direct and immediate stimulatory effect of glucagon upon insulin secretion [10] and its lack of stimulatory effect upon insulin synthesis [18]. The chronic influence of both hyperinsulinism and hyperglucagonism upon islet function could correspond, for instance, to a change in the level of those enzymes which control within the beta-cell the processes of insulin synthesis and secretion. Such a hypothesis is comparable with that evoked in order to explain the chronic effects of other hormones upon islet function [16, 11], and is substantiated by the enzyme changes described by JOHANSSON and TÄLJEDAL [5] in the islets of rats chronically treated with glucagon.

Changes in islet function comparable with those observed in experimental hyperinsulinism and hyperglucagonism could play a role in the metabolic derangements seen in patients immediately after surgical removal of an insulinoma, and in patients having a glucagon-producing tumour. In both cases, impaired insulinic response of the pancreas to glucose could account for the reduced tolerance to glucose which characterizes these clinical conditions [1, 17].

Acknowledgements. The authors wish to thank Misses SUSANNE KING and MARIANNE VANWYNGAERDEN for skilled technical assistance. This work was supported in part by a grant from the Fonds National de la Recherche Scientifique (Brussels) and by grants-in-aid from the Lilly Research Laboratories (Indianapolis) and the Novo Research Institute (Copenhagen).

References

1. FLOYD, J.C., Jr, S.S. FAJANS, R.F. KNOPF, and J.W. CONN: Plasma insulin in organic hyperinsulinism. Comparative effects of tolbutamide, leucine and glucose. J. clin. Endocrin. **24**, 747–760 (1964).
2. HAIST, R.E.: Factors affecting the insulin content of the pancreas. Physiol. Rev. **24**, 409–444 (1944).
3. HOFFMAN, W.S.: A rapid photoelectric method for the determination of glucose in blood and urine. J. biol. Chem. **120**, 51–55 (1937).
4. HOUSSAY, B.A.: Thyroid and metathyroid diabetes. Endocrinology **35**, 158–172 (1944).
5. JOHANSSON, S., and I.B. TÄLJEDAL: Stimulation of 5-nucleotidase activity in the pancreatic islets of rat by glucagon. Endocrinology **82**, 173–174 (1968).
6. LOGOTHETOPOULOS, J., B.B. SHARMA, J.M. SALTER, and C.H. BEST: Glucagon and metaglucagon diabetes in rabbits. Diabetes **9**, 278–285 (1960).
7. MALAISSE, W.: Etude de la sécrétion insulinique in vitro. Bruxelles, Editions Arscia, p. 156, 1968.
8. MALAISSE, W., and F. MALAISSE-LAGAE: Insulin secretion by isolated islets of Langerhans. Effects of pancreatic and intestinal hormones. Acta diab. latina **5** (Suppl. **1**), 64–76 (1968).
9. — — C. PICARD, and J. FLAMENT-DURAND: Effects of pregnancy and chorionic growth hormone upon insulin secretion. Endocrinology **84**, 41–44 (1969).
10. — —, and D. MAYHEW: A possible role for the adenylcyclase system in insulin secretion. J. clin. Invest. **46**, 1724–1734 (1967).
11. — —, and E.F. MCCRAW: Effects of thyroid function upon insulin secretion. Diabetes **16**, 643–646 (1967).
12. — — —, and P.H. WRIGHT: Insulin secretion in vitro by pancreatic tissue from normal, adrenalectomized,

and cortisol-treated rats. Proc. Soc. exp. Biol. (N.Y.) **124**, 924–928 (1967).
13. — —, and P.H. Wright: A new method for the measurement *in vitro* of pancreatic insulin secretion. Endocrinology **80**, 99–108 (1967).
14. — — — Effect of fasting upon insulin secretion in the rat. Amer. J. Physiol. **213**, 843–848 (1967).
15. — — —, and J. Ashmore: Effects of adrenergic and cholinergic agents upon insulin secretion *in vitro*. Endocrinology **80**, 975–978 (1967).
16. — —S. King, and P.H. Wright: Effect of growth hormone on insulin secretion. Amer. J. Physiol. **215**, 423–428 (1968).
17. McGavran, M.H., R.H. Unger, L. Recant, H.C. Polk, C. Kilo, and M.E. Levin: A glucagon-secreting alpha-cell carcinoma of the pancreas. New Engl. J. Med. **274**, 1408–1413 (1966).
18. Taylor, K.W.: Regulation of insulin biosynthesis. Excerpta Medica Foundation, International Congress Series, in press.

Dr. W. Malaisse
Laboratoire de Médecine Expérimentale
Université Libre de Bruxelles
115 Blvd. de Waterloo
Bruxelles 1, Belgique