Neuroendocrine Control of Insulin Secretion

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SUMMARY

The effects of thyrotropin-releasing hormone, luteinizing hormone-releasing hormone, substance P, somatostatin, and a partially purified hypothalamic extract on insulin secretion were tested both in vitro and in vivo. Only somatostatin and the hypothalamic extract affected insulin secretion. In vitro, somatostatin decreased glucose-stimulated insulin secretion by isolated islets and in vivo significantly reduced the rate of insulin output into the portal vein. The hypothalamic extract significantly stimulated insulin secretion in both systems. These effects in vivo were independent of glucose concentration. Islets preincubated for four hours responded better in vitro to the hypothalamic extract stimulation and the somatostatin inhibition. DIABETES *25*:96-100, February, 1976.

Neural control of insulin secretion has been demonstrated.[1] Stimulation of the sympathetic nerves inhibits insulin release, while parasympathetic nerve stimulation has the opposite effect. In addition to this neurologic control, humoral regulation of the pancreatic islets by the central nervous system has been postulated. A perifusate of the mouse lateral hypothalamus stimulates insulin release by isolated islets.[2] Similarly, an extract of the rat lateral hypothalamus increases insulin release by isolated rat islets.[3] In the monkey, electrical stimulation of the lateral hypothalamus results in an increase of islet-stimulating activity in blood collected from the internal jugular vein.[4] This islet-stimulating activity is also demonstrable in rat plasma and disappears following bilateral destruction of the ventrolateral hypothalamus.[3] The recent finding that somatostatin (a hypothalamic polypeptide) inhibits insulin and glucagon secretion[5-7] supports the concept of neuroendocrine control of the pancreatic islets.

This paper reports the results of a study in which the effects of somatostatin and a partially purified hypothalamic extract on insulin secretion were assessed both in vitro and in vivo. In addition, other hypothalamic polypeptides, namely TRH,* LHRH,† and substance P, were tested in the same systems. The reasons for this were twofold: (1) to explore whether hypothalamic hormones of known structure other than somatostatin might also affect insulin secretion, and (2) to ascertain that none of those compounds could be responsible for the stimulatory effect of the hypothalamic extract being prepared in our laboratory.

MATERIALS AND METHODS

Animals. Wistar rats in the weight range 250-300 gm. were used: for the in-vitro experiments, male rats had free access to food and water to the time of death. For in-vivo experiments, female rats were fasted overnight prior to use.

Chemicals. TRH and LHRH were obtained as pure, freeze-dried preparations from Farbwerke Hoechst A.G., Frankfurt (Main). Synthetic substance P (bovine) (lot A 0931) was purchased from Beckman Instruments, Palo Alto, and somatostatin was the kind gift of Dr. Gregory Brown (Clark Institute, Toronto). Stock solutions of each peptide (1 mM) were prepared in saline, and aliquots were stored at −20° until use. Repeated freezing and thawing were avoided. The hypothalamic extract was a partially purified preparation obtained from 200 gm. of fresh bovine hypothalami. The extraction procedure was

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*Thyrotropin-releasing hormone.

†Luteinizing hormone-releasing hormone.

similar to that reported for the isolation of neurotensin.[8] The freeze-dried extract was dissolved in 0.1 M acetic acid and fractionated through a G-25 Sephadex column (2 × 55 cm.) equilibrated with 0.1 M acetic acid. The active fraction (Ve/Vo = 1.6) was dried by flash evaporation. The residue was dissolved in 0.1 M acetic acid and subjected to paper electrophoresis at pH 6.5.[9] Peptides were visualized with ninhydrin in a guide strip and eluted from unstained paper with 0.1 M acetic acid. Nine ninhydrin-positive bands were observed, the islet-stimulating activity being recovered from one band moving anodally from the application point with Rf = 0.70 relative to aspartic acid (figure 1). For the in-vitro and the in-vivo tests the material eluted from the paper was freeze-dried and dissolved in 5 ml. saline just before the experiment. The protein content of the stock solution was 150 μg./ml.[10] The rat insulin standards for the immunoassay were a gift from Novo Laboratories, Copenhagen. All other reagents used were of the highest purity available from commercial sources.

Isolation and incubation of rat islets of Langerhans. Pancreatic islets were isolated by the technic of Lacy and Kostianovsky.[11] Half of the total number of islets, randomly selected, were transferred to a Petri dish containing fresh Hank's solution for immediate use in the incubation system described below. The remaining islets were collected in a siliconized flask containing 10 ml. of Minimal Essential Medium (Eagle) (Flow Laboratories, Rockville, Maryland) with bovine plasma albumin (1 mg./ml.) as the only addition. The islets in this medium were preincubated for four hours at 37° C. under an atmosphere of O_2 + CO_2(95:5, v/v). At the conclusion of this preincubation period the islets were washed three times with Hank's solution and were then employed in the experimental incubation system exactly as described for the freshly prepared islets. To investigate the effects on insulin release of the various hypothalamic materials in vitro, groups of five islets were incubated in 1-ml. volumes of Gey and Gey buffer[12] containing 2 mM $CaCl_2$, 1 mg./ml. of bovine plasma albumin, and 7 mM or 20 mM glucose together with the various test substances. The gas phase was O_2 + CO_2(95:5), and the pH (7.4) was checked before and after incubation, which was carried out for one hour at 37° C. with gentle shaking. Following incubation, samples of the media were taken for triplicate determination of their insulin content by radioimmunoassay.[13] None of the test substances, at the concentrations used, interfered with the recovery of standard insulin in the assay.

Experiments in vivo. Under ether anesthesia, the rat abdomen was opened and a 19-gauge indwelling catheter was introduced through the superior mesenteric vein until its tip reached the trunk of the portal vein. Blood was allowed to flow freely into heparinized, graduated centrifuge tubes. During the first 90 seconds a baseline sample was collected. At this point, 0.5 ml. of either the test substance or saline was injected into the tail vein and portal blood was collected in separate tubes for three successive periods of two, three, and five minutes following the injection. The volume of each sample was recorded and the plasma separated by centrifugation. On aliquots of the plasma samples, insulin[13] and glucose[14] were determined. The final solution of somatostatin, TRH, LHRH, and substance P was made in saline, such that each animal received 100 pmol in the injected volume of 500 μl.‡ In the case of the hypothalamic extract, 100 μl. of the stock solution was diluted with 400 μl. of saline prior to injection. The results were expressed as microunits of insulin secreted per minute by correcting the insulin concentration in each portal sample by the "flow," which was an estimate based on the volume of each sample divided by the collection time. For the 30 animals in this study, the mean "flow" (mean ± S.E.) was 0.5 ± 0.02 ml. per minute, which would represent one tenth of the total portal flow in animals of this size.[15]

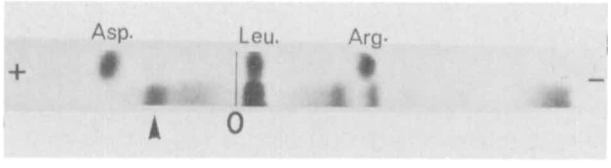

FIG. 1. Electrophoresis of hypothalamic extract from G-25 column on paper (16 cm. Whatman 3 MM) in pyridine-acetic acid-water (25:1:225, v/v), pH 6.5 at 20 v/cm. for five hours. The load was 1 mg. per centimeter. Peptides and amino acid were visualized with ninhydrin. 0: application point. Arrowhead: active fraction band.

RESULTS

Experiments in vitro. As shown in table 1, somatostatin (5 × 10^{-8}M) inhibited insulin release from freshly isolated islets incubated in the presence of both 7 mM and 20 mM glucose, though the effect attained statistical significance ($P < 0.01$) only with the lower glucose concentration. However, when islets preincu-

‡Expressed in weight, each animal was given: somatostatin, 150 ng.; TRH, 400 ng.; LHRH, 1.2 μg.; substance P, 150 ng.

TABLE 1

Effects of somatostatin, a hypothalamic extract, LHRH, TRH, and substance P on insulin released by isolated islets

Additions to medium	Insulin release (μU./5 islets/hour)			
	Fresh islets	P	Preincubated islets	P
7 mM glucose (7)	500 ± 4		521 ± 39	
7 mM glucose + 5.10^{-8} M somatostatin (7)	382 ± 35	< 0.01	285 ± 25	< 0.001
20 mM glucose (7)	1,051 ± 138		948 ± 120	
20 mM glucose + 5.10^{-8} M somatostatin (7)	828 ± 90	N.S.	432 ± 80	< 0.005
7 mM glucose (7)	432 ± 29		355 ± 31	
7 mM glucose + 10 μl. hypoth. ext. (6)	477 ± 20	N.S.	760 ± 108	< 0.005
7 mM glucose + 50 μl. hypoth. ext. (6)	626 ± 52	< 0.01	1,041 ± 108	< 0.001
7 mM glucose (10)	350 ± 39		468 ± 57	
7 mM glucose + 2×10^{-7} M LHRH (10)	347 ± 30	N.S.	548 ± 59	N.S.
7 mM glucose + 2×10^{-7} M TRH (10)	386 ± 60	N.S.	405 ± 33	N.S.
7 mM glucose + 2×10^{-7} subst. P. (10)	400 ± 63	N.S.	484 ± 59	N.S.
7 mM glucose (6)	235 ± 27			
7 mM glucose + 10^{-5} M LHRH (6)	230 ± 32	N.S.		
7 mM glucose + 10^{-5} M TRH (6)	231 ± 30	N.S.		
7 mM glucose + 10^{-5} subst. P. (6)	174 ± 22	N.S.		

Groups of five freshly prepared or preincubated islets were incubated for one hour in 1 ml. of medium containing the test substances shown. At the conclusion of incubation, samples of the media were taken for determination of their insulin content by radioimmunoassay. Results are expressed as the means of the numbers of observations in parentheses ± S.E.M. P values calculated for the result of each test substance vs. the corresponding glucose alone.

bated in nutrient medium for four hours were used, the somatostatin inhibition increased, being statistically significant at both glucose concentrations. A similar observation was made with the hypothalamic extract. In fresh islets incubated in medium containing 7 mM glucose, the addition of 10 μl. of the stock hypothalamic extract was without effect on insulin release, while a significant stimulation was observed when the concentration of the extract was increased fivefold: by contrast, when cultured islets were employed, the lower concentration of the hypothalamic preparation led to a twofold increase in the rate of insulin release above that of the control ($P < 0.005$), and the effect of the higher concentration was augmented (table 1). At a concentration of 2×10^{-7} M, LHRH, TRH, and substance P were all without significant effect on insulin release from either fresh or preincubated islets. Fiftyfold higher concentrations of these peptides were similarly inactive when tested with freshly prepared islets (table 1).

Experiments in vivo. To validate the in-vivo technic, low (10 mg./100 gm. body weight) and high (200 mg./100 gm. body weight) doses of glucose were given intravenously in 500 μl. of saline. As shown in table 2, these injections produced dose-related responses in blood glucose concentration and insulin secretion. With the high-glucose dose the insulin output was significantly higher than the baseline throughout the experiment, while the increase elicited by the low dose was significant only during the first two minutes. Both doses of glucose produced a biphasic insulin response. Of the other test substances, only somatostatin and the hypothalamic extract affected insulin secretion (table 2). Somatostatin produced a progressive decrease in insulin output that was statistically significant between five and ten min-

TABLE 2

Rates of insulin secretion and glucose concentration in the portal vein

	Insulin (μU./min.)				Glucose (mg./100 ml.)			
Time (min.)	0	0-2	2-5	5-10	0	0-2	2-5	5-10
Treatment[1]								
Saline (500 μl.) (5)	44 ± 15	39 ± 17	57 ± 24	58 ± 32	97 ± 17	112 ± 22	131 ± 31	168 ± 54
Low glucose (100 mg./kg.) (3)	108 ± 32	315 ± 52†	248 ± 90	216 ± 107	91 ± 4	126 ± 2	125 ± 8	105 ± 6
High glucose (2 gm./kg.) (3)	108 ± 31	837 ± 143*	531 ± 101†	631 ± 159‡	96 ± 3	466 ± 60	478 ± 62	361 ± 34
Somatostatin (100 pmol) (4)	44 ± 12	28 ± 15	18 ± 6	13 ± 3‡	104 ± 7	114 ± 12	121 ± 19	107 ± 15
Hypothalamic extract (15 μg. protein) (4)	24 ± 9	66 ± 26	82 ± 28‡	65 ± 16‡	105 ± 12	115 ± 7	128 ± 14	109 ± 2
LHRH (100 pmol) (4)	34 ± 8	18 ± 3	34 ± 1	26 ± 1	93 ± 7	100 ± 9	94 ± 9	120 ± 13
TRH (100 pmol) (3)	64 ± 5	48 ± 11	92 ± 31	47 ± 11	101 ± 13	121 ± 6	124 ± 16	134 ± 2
Substance P (100 pmol) (4)	51 ± 33	19 ± 12	16 ± 9	16 ± 5	76 ± 12	76 ± 17	79 ± 20	81 ± 20

[1]All substances given intravenously in 500-μl. volume of saline.
P values were calculated by the Students *t* test comparing each value vs. baseline (0 time).
*P < 0.01
†P < 0.02
‡P < 0.05

utes after injection. Administration of hypothalamic extract resulted in a continuous rise of insulin secretion that was significant after the two-minute period. In neither case could the response be attributed to changes in glucose concentration. LHRH, TRH, and substance P failed to produce any statistically significant alteration in insulin secretion.

DISCUSSION

Somatostatin and a partially purified hypothalamic extract are active in respectively inhibiting and augmenting glucose-stimulated insulin release both in vitro and in vivo. In vitro, the stimulatory action of the hypothalamic factor is demonstrable only in the presence of glucose, being maximal at glucose concentrations between 5 and 7 mM (unpublished observation). LHRH, TRH, and substance P are without effect. Although the hypothalamic extract has not been fully characterized, the observation that its effect was not reproduced by pharmacologic doses of the other hypothalamic peptides suggests that it is distinct from LHRH, TRH, and substance P. This view is also supported by the fact that both LHRH and TRH do not react with ninhydrin, while at the present state of purity the hypothalamic extract is ninhydrin-positive. In addition, the electrophoretic mobility of the various hypothalamic materials also differs: while the purified peptides tested are positively charged at pH 6.5, our hypothalamic extract moves to the anode at this pH. The opposing effects of somatostatin and the hypothalamic extract on insulin release suggest a means by which the hypothalamus might regulate the function of the pancreatic B-cells by a humoral pathway. However, in the absence of available data concerning peripheral plasma concentrations of these hypothalamic agents, the physiologic significance of such a neurohormonal mechanism in the maintenance of glucose homeostasis cannot be determined yet. Similarly, at the present state of this investigation it is not possible to ascertain whether the islet-stimulating material has an exclusive hypothalamic origin or if it shares the ubiquity of somatostatin[16,17] and TRH.[18]

The demonstration that the effects of the two active hypothalamic materials on insulin release in vitro are enhanced by preincubation of the islets in a nutrient medium for four hours prior to study confirms a recent report that islets cultured for 48 hours show enhanced sensitivity to inhibition of insulin release by somatostatin.[19] This finding could indicate, as sug-

gested by others,[20] that collagenase digestion may change the conformation of surface determinants of the B-cells such that the perception of/or the responsiveness to regulatory agents is diminished and that a period of culture may permit repair of the damage. Since culture led to enhancement of the response to both an inhibitory and a stimulatory agent, it seems unlikely that the loss of responsiveness following collagenase treatment can be attributed to derangement of the mechanisms of exocytosis. It is tempting to speculate that the collagenase digestion may alter the properties of surface receptors for the hypothalamic factors, and further study should be directed to the testing of this hypothesis.

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