The Insulin Releasing Activities of Extracts of Pork Intestine

A.J. MOODY, J. MARKUSSEN, A. SCHAICH FRIES, C. STEENSTRUP and F. SUNDBY

Novo Research Institute, Fuglebakkevej 115, Copenhagen, Denmark

W. MALAISSE and F. MALAISSE-LAGAE

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

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Summary. Acid-ethanol extracts were prepared from pork ileum + jejunum (TOT), heart and duodenum. TOT was fractionated by column chromatography. The insulin-releasing activities (IRA) of these materials were determined using rat islets and pieces of rat pancreas incubated with 16.6 mM glucose. The heart and duodenum extracts were without effect on insulin release. Pancreatic glucagon and TOT significantly increased insulin release. Synthetic secretin did not increase insulin release by isolated islets. Pancreozymin had only slight effects on the insulin output by islets. Some of the fractions of TOT increased the insulin output of islets and pancreas pieces. The effects of these fractions were concentration-dependent in the range 5 to 250 $\mu\text{g/ml}$. The contents of GLI, pancreozymin and secretin in these materials are compared with their IRAs. The IRA described here is not caused by secretin, and is probably not caused by pancreozymin. There is no quantitative correlation between the GLI and the IRA of the fractions.

Les activités insulino-libératrices des extraits de l'intestin de porc

Résumé. Des extraits à l'acide éthanol ont été préparés à partir du cœur, duodénum, ileum + jejunum (TOT) du porc et TOT fractionnée sur colonne chromatographique. Avec les îlots du rat et les morceaux de pancréas du rat, incubés dans du glucose 16.6 mM, on a déterminé pour chaque produit son activité insulino-libératrice (IRA). Les extraits de cœur et duodénum n'accrurent pas la libération d'insuline; le glucagon pancréatique et TOT l'augmentèrent significativement. La sécrétine synthétique n'agit point sur la libération d'insuline, par les îlots isolés, mais la pancréozymine très faiblement. La production d'insuline, par les îlots et les morceaux, fut stimulée par quelques fractions de TOT, les effets dépendant des concentrations des fractions

entre 5 et 250 $\mu\text{g/ml}$. Les contenus en GLI, pancréozymine et sécrétine de ces produits furent comparés avec leurs IRAs. L'IRA, rapporté ici, n'est pas due à la sécrétine, ni probablement à la pancréozymine. Il n'existe pas de relations quantitatives entre le GLI et IRA des fractions.

Extrakte aus Schweine-Eingeweiden und ihre stimulatorischen Effekte auf die Insulin-Ausschüttung

Zusammenfassung. Säureethanolextrakte wurden aus dem Ileum + Jejunum (TOT), Herz und Duodenum des Schweins zubereitet. TOT wurde mittels Säulenchromatographie fraktioniert. Die insulinfreisetzende Aktivität (IRA) dieser Stoffe wurde an Ratteninseln und Rattenpankreasstückchen bestimmt, die mit 16.6 mM Glucose inkubiert worden waren. Die Herz- und Duodenumextrakte hatten keine Wirkung auf die Insulinausschüttung. Pankreasglucagon und TOT bewirkten eine signifikante Erhöhung der Insulinfreigabe. Synthetisches Sekretin bewirkte keine Erhöhung der Insulinfreisetzung aus isolierten Inseln. Pancreozymin hatte nur eine geringe Wirkung auf die Insulinproduktion der Inseln. Einige der Fraktionen von TOT erhöhten die Insulinproduktion der Inseln und der Pankreasstückchen. Im Konzentrationsbereich von 5–250 $\mu\text{g/ml}$ waren die Wirkungen dieser Fraktionen konzentrationsabhängig. Der GLI-, Pancreozymin- und Sekretin Gehalt dieser Stoffe wird mit ihren IRAs verglichen. Die hier beschriebene IRA wird nicht durch Sekretin verursacht, wahrscheinlich auch nicht durch Pancreozymin. Es besteht keine quantitative Wechselbeziehung zwischen der GLI und der IRA der Fraktionen.

Key-words: Intestine, insulin-releasing factor, GLI, islets, pancreas pieces.

Various gastro-intestinal hormones are thought to play a role in the higher insulinic response evoked by oral as distinct from intravenous administration of glucose. The present experiments demonstrate the insulintropic effect of a gut extract on isolated rat islets and pieces of rat pancreas, and the similarity between this effect and that of pancreatic glucagon.

Materials and Methods

Tissue Extracts

1. Preparation of the crude material

185 kg of pork ileum + jejunum was flushed with cold water at the earliest possible moment after

slaughter. The material was then frozen on solid carbon dioxide. The frozen tissue was sliced into 75% ethanol containing 1.5% H_3PO_4 . The tissue was extracted for two hours with stirring, and the residue removed by centrifugation. The residue was extracted with 60% ethanol containing 0.5% H_3PO_4 . The combined extracts were filtered with kieselguhr, and the ethanol was evaporated *in vacuo*. The concentrate was filtered. Solid trichloroacetic acid (TCA) was added to the filtrate to give a final concentration of 10% w/v. The precipitate was removed by centrifugation and extracted with 50% and then with 75% acetone, whereby the TCA precipitate was almost completely dissolved. The acetone extracts were combined, and acetone added to

a final concentration of 97%. 4 N HCl was added to give a flocculent precipitate. The precipitate was isolated by centrifugation, washed twice in acetone and then dried *in vacuo*. This material was designated TOT, i.e., an extract of the *total* ileum + jejunum.

Similar preparations were made on a smaller scale from heart and duodenum, and designated heart and duodenum, respectively.

2. Fractionation of the crude material

a) *Gel Filtration*. TOT was fractionated on a 15×130 cm column of Sephadex G-50 using 1 M acetic acid as an eluent. The effluent contained two zones of high absorbance at 276 m μ : one lying between the column void volume and 0.36 of the column volume; and the other lying between 0.55 and 0.85 of the column volume. The zones were designated I and II, respectively, and the UV-absorbing materials from the zones were isolated by freeze drying.

b) *Ion Exchange Chromatography*. Fraction II was dissolved in 0.01 M tris-HCl pH 8.5 containing 0.01 M NaCl. The pH of the solution was adjusted to 8.5 with NaOH. A precipitate formed, which was removed by centrifugation. The supernatant was applied to a 10×40 cm column of DEAE-Sephadex equilibrated with 0.01 M tris-HCl (pH 8.5) containing 0.01 M NaCl. The sample was eluted with a gradient of NaCl (0.01 M to 0.2 M) in 0.01 M tris-HCl (pH 8.5). The elution was carried out at 250 ml/h. The effluent was cut into fractions corresponding either to a peak in the UV absorbance or a peak in the GLI content of the fraction. Seven fractions were prepared, designated: α , β , γ , δ , ϵ_1 , ϵ_2 and ω . The fractions were concentrated by dialysis against dry polyethylene glycol 6000. The dialysis residues were brought to 10% TCA and the precipitates dissolved in 5 ml of 50% acetone in water. The proteins were precipitated by the addition of 95 ml of acetone and 4 N HCl. The acetone precipitates were centrifuged down, rinsed twice with acetone and dried *in vacuo*.

Fraction β was further fractionated into β_1 and β_2 by LKB electrofocusing [2].

3. Content of known hormones

The glucagon-like immunoreactivities (GLI) of these fractions were determined by radioimmunoassay [3], using an anti-pork glucagon rabbit serum (K 36). GLI content is expressed in ngEqv/mg fraction, relative to standards of pork pancreatic glucagon. The pancreozymin-like immunoreactivities (PLI) of these fractions were determined by radioimmunoassay [5] using an anti-gastrin tetrapeptide rabbit serum. The PLI contents are expressed in ngEqv/mg fraction relative to standards of highly purified pancreozymin¹.

The secretin contents of the fractions were measured by a cat-bioassay [6], using Boots' Secretin as a standard.

Insulin-releasing activities

The insulin-releasing activities of the fractions were determined by incubating them with isolated rat islets and with pieces of rat pancreas. The effects of pork pancreatic glucagon (NOVO, containing less than 0.01% of insulin), synthetic secretin (Squibb, batch SQ 18, 773-ES-XXIX-109, approximate specific activity 4000 C.U./mg) and highly purified pancreozymin¹ on the release of insulin by islets were also studied.

1. Incubation of isolated islets

A bicarbonate-buffered medium equilibrated with 95% O₂: 5% CO₂ and containing human serum albumin (1% w/v), 5 mM sodium fumarate, glutamate and pyruvate [7] (fortifier) was used in the majority of the islet experiments. The medium contained 250 K.I.U. of kallikrein inhibitor/ml (Trasylool, Bayer). This drug protects polypeptides from lytic factors present in acinar tissue [9], and in blood [1], but does not modify the rate of insulin secretion induced by glucose [9].

Islets of Langerhans were isolated from the pancreases of fed, 200 or 400 g male Wistar rats by collagenase digestion [8]. The islets from two or three rats were pooled for each experiment. The pooled islets were preincubated for one hour at 37°C in medium containing 8.3 mM glucose. The islets were then transferred to ice-cold medium and distributed among the incubation flasks. Six to eight islets were added to each flask. Each flask contained 2 ml of bicarbonate medium with a glucose concentration of 8.3 or 16.6 mM. Equal numbers of flasks were prepared with glucose alone and with glucose plus the fraction to be tested. The flasks were gassed with 95% O₂: 5% CO₂ and incubated for 90 min at 37°C.

After the incubation, aliquots of the media were diluted for the assay of their insulin contents. The insulin in the media was determined in duplicate by radioimmunoassay [4]. Rat insulin (NOVO, lot R 464, potency 20 U/mg) was used as a standard.

The insulin contents of the materials tested were determined in the same system. All the values presented are corrected for the insulin added to the media with the test substances.

The results are expressed as the mean difference in insulin release (μ U/islet $\cdot$ 90 min incubation) between the flasks containing glucose alone, and the flasks containing glucose plus the fraction to be tested. The significance of the difference was determined by Student's "t" test (d.f. = $2n - 2$).

2. Incubation of pancreatic tissue

Small pieces of rat pancreatic tissue were incubated in a bicarbonate-buffered medium equilibrated with

¹ Donated by Dr. J.E. Jorpes, Karolinska Institutet, Stockholm, Sweden.

95% O₂: 5% CO₂ and containing bovine serum albumin (0.5% w/v). The medium contained 625 K.I.U. of kallikrein inhibitor/ml (Trasylol, Bayer). Anti-insulin guinea-pig serum (prepared and kindly donated by Dr. P.H. Wright, Indiana University, Indianapolis) was added to the medium as required.

The rate of insulin secretion was calculated from the partial neutralization of the antibodies, and is expressed as microunits of insulin secreted per mg tissue during 60 minutes incubation at 37°C. Full details for the method of incubation and assay of the antibodies are given elsewhere [10, 15]. In one or more experiments, insulin secretion was measured in an equal number of flasks (*n*) containing a substrate alone (glucose or leucine), and the same substrate with the added extract. Mean differences (d.f. = 2*n* - 2) in secretion rates found under these conditions are quoted, together with the mean absolute rates of secretion obtained in all experiments where only the substrate was present in the medium. Theophylline (1.4 mM) and mannoheptulose (2.5 mM) were added to the medium as indicated in the results section. Iso-osmolarity was maintained in all cases by reducing the concentration of sodium chloride.

The extracts used were a pork gut extract (TOT), a duodenum extract, a heart extract, and fractions of TOT comparatively rich in GLI (fractions β_1 and ϵ_1). Preliminary results obtained with high concentrations (1.0 mg/ml) of the gut extract [11] might correspond to a substrate effect and are, therefore, not included in the present account.

Results

The hormonal contents of the fractions tested are shown in Table 1.

Table 1. *Secretin, GLI and PLI in gut extracts and in fractions of TOT*

Fraction	Secretin C.U./mg	GLI ngEqv/mg	PLI ngEqv/mg
Duodenum	~ 0.02	0	—
TOT	0.2—0.04	280	17
I	0	0	—
II	—	600	—
α	0.2	150	11
β	1—2	1000	160
β_1	—	490	—
γ	~ 0	116	28
δ	~ 0	216	21
ϵ_1	~ 0	3500	54
ϵ_2	~ 0	1500	32
ω	~ 0	300	8

TOT contains some secretin and some GLI. Fraction II contains all the GLI present in TOT, but only about one-half of the protein. Secretin is restricted to fractions α and β of TOT. GLI is found in all the fractions derived from fraction II of TOT, with high concentrations located in β , β_1 , ϵ_1 and ϵ_2 . TOT contains 17 ngEqv of PLI/mg. Fraction β contains 160 ngEqv/mg. The other fractions derived from TOT contain between 8 and 54 ngEqv of PLI/mg.

The effects of these fractions on the release of insulin by isolated rat islets are summarized in Tables 2, 3 and 4.

At 50 μ g/ml, TOT did not stimulate insulin release in the presence of 8.3 mM glucose, but did so at 5 and 50 μ g/ml in the presence of 16.6 mM glucose (Table 2). All other incubations were carried out with 16.6 mM glucose. Unless otherwise stated, 50 μ g/ml of the substances to be tested were added to the media. The heart and duodenum extracts were without effect. Secretin was without effect at a concentration (10 ng/ml) equivalent to that added with 50 μ g/ml of fraction β . Glucagon significantly stimulated insulin release. Fraction I was inert whereas Fraction II significantly increased insulin release. Fraction α was without effect. Beta slightly increased insulin release. Gamma, δ , ϵ_1 and ϵ_2 significantly increased insulin release by about 40—70% of the release observed with glucose alone. Omega contained 220 μ U of insulin/50 μ g. The effect of this fraction on the insulin output of islets was equal to that which would be expected from its content of insulin.

Pancreozymin was examined in a separate series of experiments in which its effects were compared with those of β , β_1 and γ .

Table 3 (A and B) shows that pancreozymin at 1.5 and 10 ng/ml was without statistically significant effect on the insulin output of islets incubated in the presence of 16.6 mM glucose. In the same experiments γ at 50 μ g/ml (equal to about 1.5 ngEqv of PLI/ml) significantly stimulated insulin release.

Removal of the fortifier from the incubation medium reduced the insulin released in the presence of glucose alone (compare lines 1 and 5 in Table 3) without markedly decreasing the effect of added fractions. Pancreozymin was therefore compared with β and β_1 in buffer without added fortifier so that minor effects of these materials could be exposed.

Beta and β_1 weakly stimulated insulin release (Table 3, D). Pancreozymin at 5 ng/ml, which was approximately equal to the amount of PLI added with 50 μ g of β /ml, slightly increased insulin release ($0.025 < P < 0.05$). At 250 ng/ml pancreozymin weakly inhibited insulin release ($P = 0.001$).

Fractions γ , δ and ϵ_1 were added to islets at concentrations ranging between 5.0 and 250 μ g/ml (Table 4). Between 5.0 and 50.0 μ g/ml, the effects of all the fractions increased. There was no significant difference between the effects of the fractions in this concentration range. Between 50 and 250 μ g/ml, the

effect of γ and ϵ_1 increased, whereas the effect of δ decreased.

The results obtained by incubation of rat pancreatic tissue are summarized in Table 5.

TOT had no effect in the absence of glucose, but it significantly increased the rate of insulin secretion induced by glucose (16.6 mM). This potentiating effect progressively faded out as the concentration of the

Table 2. *The effects of extracts and fractions on the insulin output of isolated islets*

Substances tested	$\mu\text{g/ml}$	Glucose (mM)	
		8.3	16.6
A. 1. NIL		123 $\pm$ 6 (23)	561 $\pm$ 15 (188)
B. 2. Heart extract	50	—	— 56 $\pm$ 58 (40)
3. Duodenum extract	50	—	+ 19 $\pm$ 34 (38)
4. Secretin	^a	—	+ 46 $\pm$ 66 (20)
5. Glucagon	50	—	+ 396 $\pm$ 118 ^c (10)
6. TOT	5	—	+ 173 $\pm$ 36 ^c (32)
7. TOT	50	+ 13 $\pm$ 11 (13)	+ 225 $\pm$ 81 ^b (40)
8. I	50	—	+ 33 $\pm$ 50 (20)
9. II	50	—	+ 199 $\pm$ 62 ^c (20)
10. α	50	—	— 39 $\pm$ 36 (10)
11. β	50	—	+ 90 $\pm$ 26 ^a (10)
12. γ	50	—	+ 357 $\pm$ 70 ^b (10)
13. δ	50	—	+ 220 $\pm$ 23 ^c (20)
14. ϵ_1	50	—	+ 592 $\pm$ 90 ^c (20)
15. ϵ_2	50	—	+ 225 $\pm$ 29 ^c (10)
16. ω	50	—	0 (20)

The islets were obtained from 400 g rats.

A. Mean insulin output ($\mu\text{U}/\text{islet} \cdot 90 \text{ min} \pm \text{S.E.M.}$) of islets incubated with glucose alone.

B. Effects of added materials as mean changes (+ or —) in insulin output ($\mu\text{U}/\text{islet} \cdot 90 \text{ min} \pm \text{S.E.M.}$) with their statistical significances.

^a $P < 0.05$.

^b $P < 0.02$.

^c $P < 0.005$.

The number of observations is in brackets.

^d Secretin at 10 ng/ml.

Table 3. *The effect of β , β_1 , γ and pancreozymin on the insulin output of isolated islets*

Substances tested	Glucose (16.6 mM)
A 1. NIL	387 $\pm$ 16 (19)
B 2. γ (50 $\mu\text{g/ml}$)	+ 163 $\pm$ 37 ^c (20)
3. Pancreozymin (1.5 ng/ml)	+ 37 $\pm$ 20 (20)
4. Pancreozymin (10 ng/ml)	+ 44 $\pm$ 26 (20)
C 5. NIL	282 $\pm$ 18 (36)
D 6. β (50 $\mu\text{g/ml}$)	+ 38 $\pm$ 18 ^a (15)
7. β_1 (50 $\mu\text{g/ml}$)	+ 64 $\pm$ 14 ^c (15)
8. Pancreozymin (5 ng/ml)	+ 30 $\pm$ 12 ^b (19)
9. Pancreozymin (250 ng/ml)	— 30 $\pm$ 11 ^b (19)

The islets were obtained from 200 g rats and incubated in a bicarbonate-buffered medium with (A, B) or without (C, D) added fortifier.

A and C: Mean insulin output ($\mu\text{U}/\text{islet} \cdot 90 \text{ min.} \pm \text{S.E.M.}$) of islets incubated with glucose alone.

B and D: Effects of added substances as mean changes (+ or —) in insulin output ($\mu\text{U}/\text{islet} \cdot 90 \text{ min.} \pm \text{S.E.M.}$) with their statistical significances.

^a $P < 0.05$.

^b $P < 0.02$.

^c $P < 0.005$.

The number of observations is in brackets.

extract in the incubation medium was decreased from 125 to 16 $\mu\text{g/ml}$. Mannoheptulose inhibited both the stimulant action of glucose and the enhancing effect of TOT.

Over a wide range of concentrations (5 to 64 $\mu\text{g/ml}$), and at 2 glucose levels (8.3 mM and 16.6 mM), the duodenum extract exerted only minor effects, a barely significant ($0.05 < P < 0.03$) stimulant effect being observed under only one experimental condition. The heart extract did not demonstrate any stimulant effect.

The fractions rich in GLI (β_1 and ϵ_1) influenced insulin secretion in the same manner as TOT. They had little if any effect in the absence of glucose, but markedly enhanced the rate of secretion induced by glucose (16.6 mM). This potentiating action disappeared as the concentration of these fractions in the incubation medium was decreased from 10 to 2 $\mu\text{g/ml}$.

Since leucine- and glucose-induced secretion are similar in that they are both potentiated by theophylline [12], the effects of the intestinal extracts were also investigated in the presence of leucine. This amino acid was used in a high concentration (25.0 mM) previously shown to significantly increase the basal rate of insulin secretion in the present system [12]. The gut extract significantly enhanced the stimulant effect of leucine

whereas, at the same concentration (50 $\mu\text{g/ml}$), the duodenum and heart extracts were ineffective.

As shown in Table 5, the results obtained with TOT and the fractions β_1 and ϵ_1 are comparable with those obtained in previous experiments with pancreatic glucagon or theophylline.

which stimulates insulin secretion *in vitro* [12]. Duodenum and heart extracts apparently do not contain such a factor.

The patterns of insulin-releasing activities detected by isolated islets and by pieces of pancreas are remarkably similar. Both methods detected insulin-

Table 4. *The effects of different concentrations of gut fractions on the insulin output of isolated islets*

A.		B.		
Glucose	$\mu\text{g/ml}$	Fractions Tested		
16.6 mM		γ	δ	ϵ_1
1.	5	+ 97 $\pm$ 26 ^b (6)	+ 50 $\pm$ 26 ^b (6)	+ 78 $\pm$ 32 ^b (6)
2.	339 $\pm$ 13 (18)	+ 135 $\pm$ 28 ^c (6)	+ 123 $\pm$ 25 ^c (6)	+ 123 $\pm$ 38 ^b (6)
3.	250	+ 163 $\pm$ 38 ^c (6)	+ 62 $\pm$ 27 ^b (6)	+ 163 $\pm$ 44 ^c (6)

A. Mean insulin output ($\mu\text{U/islet} \cdot 90 \text{ min} \pm \text{S.E.M.}$) of islets incubated with glucose alone. The islets were obtained from 200 g rats.

B. Effect of fractions as mean increases in insulin output ($\mu\text{U/islet} \cdot 90 \text{ min} \pm \text{S.E.M.}$) with their statistical significances.

^b $P < 0.02$.

^c $P < 0.005$.

The number of observations is in brackets.

Table 5. *The effects of gut extracts, glucagon and theophylline on the insulin output of pancreas pieces*

Line	Substances tested	$\mu\text{g/ml}$	Substrate (mM)				
			NIL	Glucose (8.3)	Glucose (16.6)	Glucose (16.6) + Mannoheptulose (2.5)	Leucine (25.0)
A. 1	NIL		13.5 $\pm$ 1.4 (72)	30.7 $\pm$ 2.8 (36)	74.1 $\pm$ 3.8 (36)	23.4 $\pm$ 5.0 (18)	21.9 $\pm$ 1.1 (72)
B. 2	TOT	125			+ 28.6 $\pm$ 5.6 ^c (36)	+ 2.4 $\pm$ 6.6 (18)	
3	id.	63	+ 3.5 $\pm$ 2.7 (36)		+ 19.5 $\pm$ 4.9 ^c (36)		
4	id.	50					+ 10.4 $\pm$ 3.1 ^b (18)
5	id.	31	+ 0.3 $\pm$ 3.2 (36)		+ 15.1 $\pm$ 7.7 (18)		
6	id.	16	- 4.7 $\pm$ 3.5 (18)		+ 12.7 $\pm$ 11.1 (18)		
7	Duodenum extract	64		+ 8.9 $\pm$ 3.9 ^a (36)			
8	id.	50			+ 9.9 $\pm$ 5.4 (54)		+ 3.2 $\pm$ 2.4 (36)
9	id.	32		+ 4.9 $\pm$ 3.6 (36)			
10	id.	16		+ 6.0 $\pm$ 3.9 (36)			
11	id.	5			+ 0.7 $\pm$ 12.4 (18)		
12	Heart extract	50			- 2.5 $\pm$ 5.2 (54)		+ 2.3 $\pm$ 2.7 (36)
13	β_1	10	+ 5.3 $\pm$ 3.6 (18)		+ 41.5 $\pm$ 10.1 ^c (18)		
14	id.	2			+ 19.2 $\pm$ 9.7 (18)		
15	ϵ_1	10	+ 7.7 $\pm$ 3.4 ^a (18)		+ 39.4 $\pm$ 8.7 ^c (18)		
16	id.	2			+ 12.2 $\pm$ 8.9 (18)		
17	Pancreatic glucagon	10	+ 3.1 $\pm$ 4.4 (9)	+ 20.1 $\pm$ 4.2 ^c (27)	+ 35.9 $\pm$ 8.2 ^c (18)		+ 6.7 $\pm$ 2.7 ^b (36)
18	Theophylline	250	- 2.6 $\pm$ 6.0 (9)	+ 31.8 $\pm$ 4.7 ^c (36)	+ 57.7 $\pm$ 8.5 ^c (36)		+ 11.4 $\pm$ 2.0 ^c (18)

A. Mean insulin output ($\mu\text{U/mg} \cdot 60 \text{ min} \pm \text{S.E.M.}$) by tissue incubated in media containing only substrates.

B. Effects of added materials are quoted as mean changes (+ or -) in insulin output ($\mu\text{U/mg} \cdot 60 \text{ min} \pm \text{S.E.M.}$) with their statistical significances.

^a $P < 0.05$.

^b $P < 0.02$.

^c $P < 0.001$.

The number of observations is in brackets.

Discussion and Conclusions

The present results confirm previous reports suggesting that the pork ileum + jejunum contain a factor

releasing activity in the extract of jejunum + ileum (TOT), and did not detect insulin-releasing activity in the extracts of heart and duodenum. The methods differ in that the insulin release from pancreas pieces was

strongly stimulated by β_1 , whereas the insulin release from islets was weakly increased by β_1 . This difference may be due to the two tissues having different sensitivities to the constituents of β_1 .

The material responsible for the enhancement of glucose-mediated insulin release by islets is not secretin, for the most active fractions were devoid of secretin, and secretin itself did not increase insulin release.

The fractions which contain the most insulin-releasing activity contained very little PLI. Furthermore, the fraction containing the most PLI was a weak stimulator of the release of insulin by islets, and pancreozymin itself was either a weak stimulator or a weak inhibitor of insulin release. It is concluded that pancreozymin is probably not the cause of the insulin releasing activities detected by the use of isolated islets. The small amounts of PLI present may have modified the insulin-releasing activities of other components of the fractions.

Our data do not permit us to decide whether the material possessing GLI is associated with, or is the causative agent of insulin-releasing activity. Insulin-releasing activity follows GLI through the purification steps, but some active fractions (γ and δ) contain little GLI whereas β , which is rich in GLI, has but little insulin releasing activity on islets. Beta₁, a GLI-containing subfraction of β , contained significant amounts of insulin-releasing activity on both islets and pancreas pieces.

The modes of action of TOT and the fractions derived from it on pancreas pieces are comparable with those previously observed with pancreatic glucagon [13]. In both cases the stimulant action was dependent upon the presence of a substrate, either glucose or leucine, in the incubation medium. It is therefore possible that the gut factor, whatever its nature (gut GLI, incretin), activates the β -cell's adenylcyclase as does pancreatic glucagon. The inhibition by mannoheptulose of the stimulant action of glucose and the potentiating effect of the gut factor is another argument in favour of such a hypothesis. Indeed, this pattern is only observed with drugs acting through the β -cell's adenylcyclase/3',5' cyclic AMP/phosphodiesterase system [13], and is different from that found with other insulinotropic agents, such as the non-carbohydrate metabolites [12] or the sulphonylureas [14].

It is concluded that an extract of pork ileum + jejunum contains a factor, or factors, which enhance glucose-induced insulin release by rat islets and pieces of rat pancreas *in vitro*. This activity is not caused by secretin and is probably not caused by pancreozymin. The insulin-releasing activity of fractions of the gut

extract is associated with the presence of GLI. The mode of action of the extract shows similarities to that of pancreatic glucagon.

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Dr. A.J. Moody
Novo Research Institute
Fuglebakkevej 115
Copenhagen/Denmark