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Insulin, Glucagon, and Somatostatin Secretion by Cultured Rat Islet Cell Tumor and Its Clones[1] (41762)

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Abstract. Cells derived from rat islet tumor and grown in culture (parent cells—RIN-m) and two clones obtained from them were used to study the effect of various secretagogues on insulin, glucagon, and somatostatin secretion. Parent cells secreted all three hormones in various quantities, while clone 5F secreted predominantly insulin and clone 14B secreted predominantly somatostatin. The secretory behavior of these cells were compared to each other and to that of normal islets. In general, as in the case of normal islets, insulin secretion was stimulated by calcium, potassium, tolbutamide, theophylline, and glucagon. It was inhibited by somatostatin. Glucagon secretion was stimulated by calcium, arginine, and theophylline. Somatostatin secretion was stimulated in clone 14B by arginine, tolbutamide, theophylline, and insulin. These cells differ from normal islets, in that they do not respond to glucose or arginine with increased insulin secretion. Also somatostatin failed to inhibit glucagon secretion. The similarity in insulin secretory responses of parent cells and clone 5F suggests that local or paracrine islet hormone secretion plays only a negligible role in the control of other hormone secretion in these cells.

A rat tumor of the islets of Langerhans has been grown in culture and cloned as described in the laboratories of Gazdar and Chick (1). The parent cell line (RIN-m) secretes relatively large quantities of both insulin and somatostatin, clone 5F secretes primarily insulin and clone 14B secretes primarily somatostatin. A single clone (clone 41) secreting glucagon has also been described (2). The fact that cloned cells secrete more than one hormone suggests cosecretion of several hormones from a single cell type, though this has not been demonstrated. However, the establishment of a growing tumor cell line producing insulin (1, 3), glucagon and somatostatin (1, 3) is an important step forward, since it could provide a tool to study certain properties of normal islet cells which have hitherto been difficult to elucidate. In order to use this tool effectively it is important to characterize the secretory behavior and responsiveness of growing tumor cells.

This report describes certain features of the hormone secretory responses of the parent cell line and of its clones to a variety of secretagogues, which have previously been studied in normal rat islet or pancreas preparations.

Materials and Methods. The establishment of continuous cell lines from transplantable rat islet cell tumor and the isolation of insulin, somatostatin, and glucagon secreting clones have been described previously (1–3). In the present study the parent cell line, RIN-m; a predominantly insulin secreting clone (clone 5F) and a predominantly somatostatin secreting clone (clone 14B) have been used. The cells were grown to confluency in 60-mm Falcon petri dishes in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum without addition of antibiotics. The medium was changed every 3 days and on the day prior to use.

Petri dishes containing $8–15 \times 10^7$ cells were used for the study. This number of cells represented a monolayer which permitted even exposure of cells to the nutrients. When the cells were confluent, 10 ml of fresh medium, containing the secretagogues to be tested were added to the cells and incubated at 37°C in a water-jacketed CO_2 incubator. Triplicate dishes were incubated for each secretagogue and respective controls. After 2 hr of incu-

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bation 2 ml of medium were sampled, added to 0.1 ml of Trasylol (FBA Pharmacia), rapidly frozen, and stored at −20°C until hormone content was analyzed. In certain experiments, 2-ml aliquots were also collected at 6 and 24 hr. The cells were then trypsinized (to prevent clumping) and counted in a Neubauer chamber. Insulin, glucagon, and somatostatin were measured by radioimmunoassay, as previously described (3). Hormone secretion was expressed per 10^7 cells. Statistical analyses were carried out using the Student *t* test. Cells were incubated with the following test substances: glucose 16.5 m*M*, arginine 19.2 m*M*, Ca^{2+} 0.8 m*M*, K^+ 1.34 or 10.7 m*M*, theophylline 1.0 m*M*, tolbutamide 0.37 m*M*, insulin 1.6×10^{-7} *M*, glucagon 2.8×10^{-6} *M*, and somatostatin 6.25×10^{-6} *M*. None of these agents altered the pH of the medium which was maintained by CO_2 in the incubation chamber. The concentrations of glucose, K^+ and Ca^{2+} in the RPMI-1640 medium were 11, 5.37, and 0.4 m*M*, respectively. The concentrations of insulin, glucagon, and somatostatin used in our study were in the pharmacologic range but were required in this system, since lower concentrations were without effect. For example, stimulation of insulin secretion occurs between 10^{-7} and 10^{-6} *M* glucagon while 10^{-11}–10^{-8} *M* concentrations produced no statistically significant insulin release. Similarly, preliminary data indicated that $0.6–6.0 \times 10^{-6}$ *M* somatostatin is required to see inhibition of insulin secretion. In rat islet cell tumor, Sopwith *et al.* (4) required $1.0–5.0 \times 10^{-6}$ *M* somatostatin to block insulin release. Further, though plasma insulin concentrations in normal dogs range from 10 to 25 μu/ml, Weir *et al.* (5) have calculated that the concentration of insulin in the proximity of the islet can be as high as 100,000 μu/ml. For this reason, large amounts of exogenous insulin were utilized in these studies.

Results. Cells from passages 8 through 30 were used. The quantitative pattern of basal hormone secretion did not change significantly during the 2-year course of this study. This was established by examination of secretion data obtained during the first year as compared with data obtained in the second year of the study.

Figure 1 shows a 24-hr time-course of basal or unstimulated hormone secretion by the parent cells and by two clones. Secretion of insulin, glucagon, and somatostatin increased with time up to 6 hr, with most of the secretion occurring during the first 2 hr. As a result, the effect of various secretagogues on hormone secretion by the parent cell line and by the clones was measured over a 2-hr period (Table I). The mean (±SEM) of the unstimulated hormone secretion for each experiment are taken as controls and the data, with addition of secretagogues, are presented as per cent of these respective unstimulated controls. The levels of significance are also indicated in Table I.

In view of the relatively small amount of immunoreactive glucagon (IRG) secreted, the identity of this material was verified by gel chromatography and immunodilution. The molecular size of IRG acid extracted (6) from pools of clones and parent cells, was identical to that of porcine glucagon standards (2). Im-

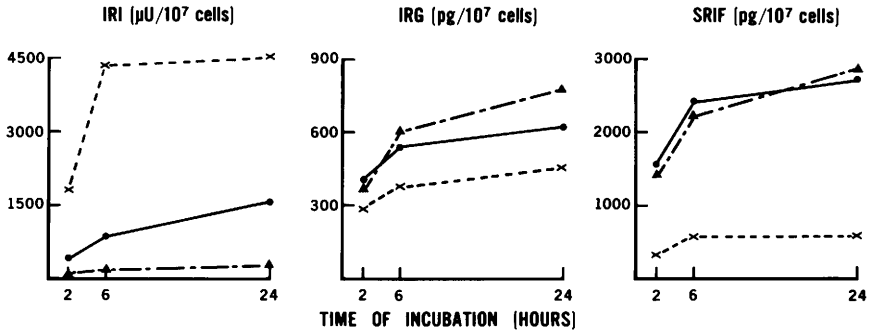

FIG. 1. Time course of basal hormone secretion from parent cells (●), clone 5F (×) and clone 14B (▲). At 2, 6, and 24 hr aliquots were removed for hormone assay. The values are the means of 18 separate experiments.

munodilution of medium IRG was similar to that of porcine glucagon (data not shown).

Elevated levels of *calcium* (0.8 m*M*) increased insulin secretion in parent and in both clones. The effect was most striking in clone 5F. Glucagon secretion increased in clone 14B. There was no significant effect on somatostatin secretion. *Glucose* (16.5 m*M*) had no effect on insulin or somatostatin secretion by parent cells or clones and did not inhibit glucagon secretion in any cell line. In fact, glucose increased glucagon secretion in clone 14B. *Arginine* (19.2 m*M*) had no effect on insulin secretion, but increased glucagon and somatostatin secretion from clone 14B. *Tolbutamide* (0.37 m*M*) increased insulin secretion from all cells. It had no significant effect on glucagon secretion from any cells, but stimulated somatostatin secretion from clones 5F and 14B. *Theophylline* (1 m*M*) stimulated insulin release markedly from parent cells and both clones and increased glucagon and somatostatin secretion from clone 14B significantly. *Glucagon* (2.8×10^{-6} *M*) stimulated insulin secretion from the parent cells and clone 5F. Glucagon also significantly inhibited somatostatin secretion from clone 14B. *Insulin* (1.6×10^{-7} *M*) had no effect on glucagon secretion from any of the cells but stimulated somatostatin secretion in the clones. *Somatostatin* (6.25×10^{-6} *M*) inhibited insulin secretion in parent cells and clone 5F but had no effect on glucagon secretion.

Elevated levels of potassium (10.7 m*M*) increased insulin secretion from parent cells and clone 5F. It increased glucagon secretion significantly from clone 5F while decreasing glucagon secretion in the parent cells. *Decreased potassium* (1.34 m*M*) had no significant effect on hormone secretion from any of the cell lines.

Discussion. A number of conclusions can be drawn from the data. The first is that this islet cell tumor differs in certain secretory responses from normal islet tissue. The most important difference is that glucose did not stimulate insulin and somatostatin secretion, nor did it suppress the secretion of glucagon. Inability of clone 5F to respond to a glucose stimulus has recently been reported (10), though in a later report Praz *et al.* (11) showed a stimulation of insulin release when glucose concentration was raised from 0 to 1.4 m*M*.

TABLE I. EFFECTS OF STIMULATORS AND INHIBITORS ON INSULIN, GLUCAGON, AND SOMATOSTATIN SECRETION BY TUMOR CELLS AND CLONES

Cells	Insulin			Glucagon			Somatostatin		
	P	5F	14B	P	5F	14B	P	5F	14B
Control†	400 ± 56‡	1810 ± 385	133 ± 19	299 ± 46	278 ± 32	428 ± 57	1460 ± 102	201 ± 21	1430 ± 251
↑ Calcium	144.7 ± 20.2*	154.6 ± 11.4*	244.3 ± 26.8§	119.3 ± 23.8	145.7 ± 26.8	152.5 ± 11.0*	122.4 ± 23.1	94.3 ± 11.7	116.3 ± 9.3
Glucose	114.9 ± 8.3	89.4 ± 15.6	134.5 ± 21.0	101.4 ± 5.9	121.0 ± 26.5	155.7 ± 10.3*	105.6 ± 9.2	119.0 ± 16.3	104.0 ± 6.7
Arginine	84.8 ± 12.3	109.9 ± 17.1	139.8 ± 23.6	118.2 ± 17.9	123.6 ± 18.1	155.7 ± 14.6*	91.7 ± 10.5	101.4 ± 3.1	170.4 ± 9.6*
Tolbutamide	135.5 ± 25.2	154.6 ± 10.8*	360.3 ± 54.0§	139.0 ± 12.9	113.8 ± 11.0	105.0 ± 5.7	120.9 ± 15.6	148.6 ± 10.3*	162.8 ± 9.1*
Theophylline	231.4 ± 27.2*	142.2 ± 20.3*	220.0 ± 37.1*	110.6 ± 8.2	117.7 ± 3.8	195.1 ± 12.3§	85.1 ± 3.6	144.7 ± 34.4	165.1 ± 7.6*
Insulin	—	—	—	88.0 ± 10.6	106.5 ± 8.1	119.7 ± 9.3	107.2 ± 17.4	183.0 ± 14.3*	159.1 ± 17.2
Glucagon	280.2 ± 46.6*	175.4 ± 9.0*	124.3 ± 17.6	—	—	—	109.7 ± 20.5	101.1 ± 0.9	74.7 ± 6.7*
Somatostatin	68.7 ± 2.9*	62.4 ± 4.8*	96.0 ± 2.2	105.4 ± 14.8	89.4 ± 12.6	122.4 ± 10.1	—	—	—
↑ Potassium	143.5 ± 20.2*	134.8 ± 10.0*	115.3 ± 9.3	61.8 ± 9.4*	150.1 ± 19.6*	121.7 ± 12.0	93.0 ± 23.7	114.3 ± 10.2	97.2 ± 6.7
↓ Potassium	104.1 ± 14.5	76.8 ± 22.8	100.1 ± 14.7	129.4 ± 15.7	90.3 ± 10.5	105.7 ± 12.6	125.0 ± 14.1	106.1 ± 7.6	85.3 ± 9.6

† Values for control are represented as $\mu u/10^7$ cells for insulin and as $pg/10^7$ for glucagon and somatostatin. Values for all other agents are represented as the percentage of respective controls run with each experiment.

‡ Mean ± SEM. All values are from three to six experiments for each agent, with triplicate determination in each experiment.

* $P < 0.05$.

§ $P < 0.01$.

They failed to show any further stimulation at glucose concentrations from 5.5 to 33 m*M* (11). When the fragments of the tumor, from which these cells were derived, were perifused with high glucose alone, no stimulation of insulin secretion was observed, though in primary monolayer cultures of these tumor cells, insulin secretion was apparently stimulated by glucose (12). It is likely that this insensitivity to glucose is due to the intrinsic characteristics of the tumor, also seen with human insulinomas (7), hamster insulinomas in culture (8, 9), and this rat insulinoma prior to culture (4). On the other hand the role of *in vitro* culture techniques must also be considered. Other significant differences from normal islets include the failure of somatostatin to inhibit glucagon secretion, and of arginine to stimulate insulin secretion, though an insulin response to the latter has been reported by others (11).

The similarities between secretory responses of this tumor and normal islets are evident in the stimulation of *insulin secretion* by calcium, potassium, tolbutamide, glucagon, and theophylline and in the *inhibition* of *insulin* secretion by somatostatin. Additional similarities include the increases in somatostatin with arginine, tolbutamide, and theophylline and the responses of glucagon to calcium and arginine.

Other characteristics of these hormone secretory responses needing further clarification and documentation include: (i) the differences in glucagon responses of parent and clones to K^+, (ii) the increased secretion of glucagon in response to glucose, and (iii) the stimulation of somatostatin secretion by pharmacologic doses of insulin.

It is noteworthy that insulin responses of the parent cell line and of the insulin secreting clones were similar, despite the fact that parent cells were simultaneously secreting somatostatin. This should not surprise us since exogenous somatostatin required to inhibit insulin release was of the order of 10^{-6} *M*. This data suggests that local islet hormone secretion (paracrine) plays a negligible role in control of other hormone secretion, at least in this tumor.

These studies also indicate that investigations dealing with the role of ions such as Ca^{2+} and K^+ or of cyclic AMP, adenylate cyclase, and of phosphodiesterase may yield information applicable to normal mechanisms of hormone secretion, though it should be remembered that in most instances tumor cells are a poor model for physiologic studies.

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