Ambiguous Effects of Colchicine and Vincristine upon A₂-Cell Response to Arginine

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Summary. In order to document the cytophysiology of glucagon release by A₂ cells, isolated perfused rat pancreases were stimulated by arginine after exposure to colchicine, vincristine, cytochalasin B, cycloheximide and puromycin. Colchicine, 10⁻⁴ mol/l, enhanced the early phase of glucagon release after a short exposure time (35 min). This potentiation vanished with longer exposure (45 to 100 min) and was replaced by a reduction in glucagon release, affecting both phases of secretion, but more the second phase than the first. When exposure time was kept constant (45 min) and the concentration of colchicine varied, a similar potentiating effect was observed for colchicine 10⁻⁷ mol/l (first phase) and 10⁻⁶ mol/l (both phases); an inhibitory influence was apparent with higher concentrations (10⁻⁵ to 10⁻³ mol/l) affecting mainly the second phase of release. Similar results were obtained with vincristine, 10⁻⁵ mol/l (infused for 35 to 100 min) and vincristine 10⁻⁸ to 10⁻⁴ mol/l, after a constant 45 min exposure. These findings are compatible with a participation of microtubules in the dynamics of glucagon release: a reduction of both phases of secretion being caused by an extended disruption in the microtubular apparatus, whereas more limited disturbance of this system is associated with facilitated glucagon release. Cytochalasin B, 10 µg/ml, potentiated the A₂ cell response to arginine and the basal glucagon release in the presence of glucose 4.4 mmol/l. Puromycin 100 µg/ml and cycloheximide 0.5 mg/ml reduced selectively the second phase of glucagon release; cycloheximide, 1 mg/ml, completely inhibited the basal release, and the early and late phases of the arginine-stimulated release. These results suggest that emiocytosis in A₂ cells is very similar to that in B cells. The specificity of antitubulins and other agents as dissecting tools for the secretory process depends upon the experimental conditions.

Key words: Colchicine, vincristine, puromycin, cycloheximide, cytochalasin B, microfilaments, microtubules, glucagon, A₂ cell.

A microtubular-microfilamentous system is present in pancreatic A₂ cells [1–5]. It is generally accepted that glucagon secretion occurs by the process of emiocytosis and that microtubules and microfilaments may be involved in this process [4–6] as well as in the B cells [7–9] and other endocrine cells [10–13]. Among the few studies devoted to the possible interference of mitotic spindle inhibitors, and microfilament inhibitors, with the corresponding organelles in the A₂ cells [14–19], there were some discrepant results: colchicine and vinblastine increased the basal and arginine-stimulated glucagon release from guinea-pig islets incubated in vitro [14], and the arginine-stimulated (but not the basal) glucagon release from fragments of rat pancreas [15]. In contrast, colchicine and vincristine were found to be inhibitors rather than stimulators in the perfused rat pancreas under certain conditions [16, 17]. Similarly, cytochalasin B enhanced both basal and stimulated release from monolayer cultures in one study [18] and the stimulated release only in another [15].

Because both inhibitory and stimulatory effects have been demonstrated for colchicine and the vinca-alkaloids on the B cell, according to concentration and time of exposure [20, 21], it appeared worthwhile to study the influence of these agents on A₂ cells, using variable concentrations and times of exposure. The isolated perfused rat pancreas was used to study the dynamics of glucagon release, and arginine was chosen for standard A₂ cell stimulation.

Material and Methods

Perfusion Technique

Male Wistar rats weighing 300–350 g, fed ad libitum with commercially available food and water, were used in all experiments. After an overnight fast, the animals were anaesthetized with pentobarbital (50 mg/kg IP). The pancreas was isolated with the proximal portion of the duodenum and separated from the spleen and stomach. Polyethylene cannulae were inserted into the abdominal aorta and portal vein respectively: the proximal part of aorta was then ligated and cut under the diaphragm; the pancreas + duodenum block was immediately placed in an incubator and perfused at a constant temperature of 37°C without recycling the medium. The flow rate was regularly measured and was maintained between 3.5 and 3.8 ml/min. A third catheter, inserted in the duodenal lumen, allowed drainage of pancreatic and duodenal exocrine secretions and avoided intra-canalicular over-pressure [22, 23].

The perfusate contained the following components: NaCl, 120 mmol/l; KCl, 4.7 mmol/l; MgSO₄, 0.8 mmol/l; CaCl₂, 2.5 mmol/l; KH₂PO₄, 1.2 mmol/l; NaHCO₃, 25 mmol/l, human serum albumin: 2.5 g/l (Centre National de Transfusion Sanguine, Paris, France) and Trasylol: 2000 anti-protease units/ml. Glucose (4.4 mmol/l) was added in all experiments. Before perfusion the medium was warmed to 37°C and gassed with a mixture of O₂ + CO₂ (95:5); final pH, 7.4. After an equilibration period of at least 20 min, L(+)-arginine, 25 mmol/l, was infused into the circuit, immediately above the pancreas, using an infusion pump (Braun, Melsungen, Germany) at a constant flow rate of 0.3 ml/min for 40 min. The complete effluent was collected from the cannula in the portal vein, at appropriate intervals, cooled on ice and frozen for storage at –20°C until assay.

Colchicine (Prolabo, Paris, France), vincristine (Eli Lilly, Indianapolis, USA), puromycin (Nutritional Biochem. Corp., Cleveland, Ohio, USA) and cycloheximide (Calbiochem, San Diego, California, USA) were dissolved in perfusate immediately before the experiments. Cytochalasin B (Calbiochem) was first dissolved in dimethyl-sulphoxide (DMSO, Regis Chemical Corp., Chicago, Illinois, USA), then diluted in perfusate (10 µg/ml): these latter experiments were compared with appropriate controls perfused with DMSO, in absence of cytochalasin. Five different concentrations were used for colchicine: from 10^{–7} to 10^{–3} mol/l and vincristine: from 10^{–8} to 10^{–4} mol/l; three concentrations were tested for cycloheximide: from 0.2 to 1 mg/ml and one con-

centration only for puromycin: 100 µg/ml, and cytochalasin B: 10 µg/ml. Each of these drugs was introduced in the circuit as soon as the aorta was cannulated (zero time) then continued until the end of the experiment. The exposure time (from time zero to the stimulation by arginine) was 45 min for all drugs. Furthermore, colchicine 10^{–4} mol/l and vincristine 10^{–5} mol/l were tested after five different times of exposure, from 35 to 100 min.

Immunoassay Procedure

Glucagon was measured by radioimmunoassay as described previously [24]. The antiserum used in this assay binds to the N-terminal part of the glucagon molecule [25] and cross-reacts with glucagon-like immunoreactivity of enteric origin commonly denominated GLI; this seemed justifiable since the GLI content of the duodenum is negligible [26]; the perfusate and all chemicals tested were added to the buffer of standard curves in order to rule out possible interference in the immunoassay. The purified glucagon standard was provided by Dr. Schlichtkrull (Novo Research Institute, Copenhagen, Denmark).

Presentation of Results and Statistical Analysis

The hormone release rate was calculated from the measured flow rate and hormone concentration. For each individual experiment planimetry of glucagon release was performed, and glucagon response to arginine obtained by subtraction of the basal release. A clear-cut separation between the first and second phases of secretion was observed, allowing calculation of the respective outputs for the early (0–5 min) and second (5–40 min) phases of response. The average was calculated for the corresponding groups. Statistical analysis was performed by Student's t test for unpaired groups [27].

Results

Basal Release and the Influence of L(+)-Arginine

The basal glucagon release, in the presence of glucose 4.4 mmol/l was 0.7 ± 0.1 ng/min. No significant variation of basal level occurred from the 10th to the 90th minute of perfusion (Fig. 1). This basal release varied substantially from experiment to experiment, but not during the course of a single perfusion. The average glucagon release rates corresponding in control perfusions to the prestimulatory, stimulatory and post-stimulatory phases were not significantly different. The individual variations of

Fig. 1. Basal glucagon release ($n = 30$) and arginine-stimulated release ($n = 9$) from the perfused rat pancreas. These preparations were perfused with glucose 4.4 mmol/l in perfusate. Zero time means the starting of perfusion immediately after catheterization of the vessels and isolation of the pancreas. Results are presented as mean values $\pm$ SEM

Fig. 2. Influence of the exposure time to colchicine on the A₂ cell response to arginine. Control data (not exposed to colchicine) are presented in the left panel for comparison ($n = 9$). Infusion of colchicine was started immediately after catheterization of the vessels (zero time). After a 35 min exposure to colchicine 10^{-4} mmol/l ($n = 3$) the first phase of release (0–5th min of the stimulation by arginine) was significantly enhanced ($p < 0.05$). After a 60 min exposure ($n = 4$), both phases of arginine-stimulated glucagon release were significantly inhibited ($p < 0.001$). Same presentation as in Figure 1

the baseline made little difference to the final calculations in view of the high rise of glucagon release observed during amino-acid stimulation. L(+)-Arginine, 25 mmol/l, strongly stimulated glucagon release. The peak response for the first phase occurred within one minute; it decreased by fourfold after 3 min, remaining however above the basal level. A sustained second phase of release was observed after five minutes. A prompt return to basal levels was observed when arginine infusion was stopped. It was verified that A₂ cell response to L(+)-arginine was not significantly different when the amino-acid was introduced into the circuit 45, 60 and 75 min after the start of perfusion.

Influence of Perfusion Time for Colchicine 10^{-4} mol/l

When colchicine 10^{-4} mol/l was perfused for 35 to 100 min (Table 1 and Fig. 2), the shortest exposure time (i. e. 35 min) was followed by a significant in-

crease of the first phase of response to arginine; the second phase, was not significantly augmented. This potentiating effect on the first phase was replaced by a significant inhibition of the second phase after 45 min and longer exposures. Both phases of glucagon release were inhibited after 60 min and longer exposures. The basal glucagon release remained unaffected in these experiments.

Influence of Variable Colchicine Concentration

After a constant perfusion time of 45 min, colchicine 10^{-7} mol/l significantly enhanced basal release and the early phase of the arginine-stimulated glucagon release. At a concentration of 10^{-6} mol/l, it significantly potentiated the early and second phases of A₂ cell response to arginine. Furthermore, basal release was significantly increased (Table 2). This potentiating effect vanished when higher concentrations were tested, and was replaced by a significant inhibition of both phases at concentrations of 10^{-4} mol/l and 10^{-3} mol/l (Table 2).

Table 1. Influence of the duration of exposure to colchicine 10⁻⁴ mol/l on arginine-stimulated glucagon release by the isolated perfused pancreas

Duration of colchicine exposure min	n	Basal release (ng/min)	Arginine-stimulated release (ng)	
			0–5th min	5–40th min
0	9	0.7 ± 0.1	9.4 ± 0.1	59 ± 10
35	3	0.6 ± 0.1	15.0 ± 4.8 ^a	74 ± 17
45	5	0.7 ± 0.1	10.8 ± 1.4	25 ± 5 ^b
60	4	0.4 ± 0.1	5.9 ± 0.6 ^b	6 ± 1 ^b
80	5	0.5 ± 0.1	2.7 ± 0.5 ^b	2 ± 1 ^b
100	4	0.5 ± 0.0	1.2 ± 0.1 ^b	2 ± 1 ^b

^a p < 0.05^b p < 0.001**Table 3.** Influence of the duration of exposure to vincristine 10⁻⁵ mol/l on the glucagon release by the isolated perfused rat pancreas

Duration of exposure min	n	Basal release (ng/min)	Arginine-stimulated release (ng)	
			0–5th min	5–40th min
0	9	0.7 ± 0.1	9.4 ± 0.9	59 ± 10
35	3	1.1 ± 0.2	12.8 ± 0.3	34 ± 2
45	4	0.7 ± 0.1	7.4 ± 2.9	22 ± 2 ^a
60	3	0.6 ± 0.2	6.5 ± 0.2	17 ± 2 ^a
80	4	0.7 ± 0.1	3.5 ± 0.6 ^b	
100	3	0.7 ± 0.1	3.0 ± 0.5 ^b	

^a p < 0.05^b p < 0.005**Table 2.** Effect of colchicine concentration on glucagon release by the isolated perfused pancreas (colchicine treatment was started 45 min prior to stimulation by arginine 25 mmol/l)

Concentration of colchicine mol/l	n	Basal release (ng/min)	Arginine-stimulated release (ng)	
			0–5th min	5–40th min
0	9	0.7 ± 0.1	9.4 ± 0.9	59 ± 10
10 ⁻⁷	3	1.0 ± 0.1 ^c	13.9 ± 2.3	77 ± 5
10 ⁻⁶	3	1.4 ± 0.1 ^b	16.8 ± 0.4 ^a	125 ± 8 ^a
10 ⁻⁵	4	0.6 ± 0.1	12.5 ± 2.5	33 ± 5
10 ⁻⁴	12	0.5 ± 0.1	6.3 ± 1.1 ^a	24 ± 4 ^b
10 ⁻³	4	0.9 ± 0.3	5.0 ± 1.0 ^a	20 ± 2 ^a

^a p < 0.05^b p < 0.005^c p < 0.001**Table 4.** Effect of vincristine concentration on glucagon release by the isolated perfused pancreas (vincristine administration was started 45 min prior to the stimulation by arginine 25 mmol/l)

Concentration of vincristine mol/l	n	Basal release	Arginine-stimulated release (ng)	
			0–5th min	5–40th min
0	9	0.7 ± 0.1	9.4 ± 0.9	59 ± 10
2.10 ⁻⁸	6	0.7 ± 0.2	12.7 ± 1.3 ^a	59 ± 6
2.10 ⁻⁷	4	0.6 ± 0.1	16.1 ± 3.2 ^a	54 ± 8
2.10 ⁻⁶	6	0.8 ± 0.2	23 ± 3 ^c	142 ± 31 ^b
10 ⁻⁵	4	0.5 ± 0.3	7.4 ± 2.9	22 ± 2 ^a
10 ⁻⁴	3	0.5 ± 0.1	4.1 ± 2.1 ^a	16.5 ± 2.1 ^a

^a p < 0.05^b p < 0.01^c p < 0.001

Vincristine: Influence of Exposure Time

When vincristine 10⁻⁵ mol/l was perfused for 35 min, no significant effect was detectable. A significant inhibition of the second phase of A₂ cell response to arginine appeared after 45 min and longer exposures; the first phase of response was inhibited only after 80 to 100 min exposures (Table 3).

Influence of Vincristine Concentration

After a constant perfusion time of 45 min, the first phase of A₂ cell response to arginine was significantly enhanced by vincristine 2.10⁻⁸, 2.10⁻⁷ and 2.10⁻⁶ mol/l (Table 4 and Fig. 3). The second phase of release was also augmented in the presence of vincristine 2.10⁻⁶ mol/l. An inhibitory effect appeared, affecting the second phase of release at

10⁻⁵ mol/l and higher concentrations, with inhibition of the first phase as well at 10⁻⁴ mol/l.

Influences of Cytochalasin B and of the Protein-Synthesis Inhibitors

The basal release of glucagon was significantly higher in the presence of cytochalasin B than in the presence of DMSO alone, or in untreated controls. Furthermore, both phases of the A₂ cell response to arginine were significantly enhanced (Table 5).

Cycloheximide, 0.5 mg/ml, and puromycin, 0.1 mg/ml, perfused for 45 min, selectively inhibited the second phase (Table 5), while the early phase was not significantly affected. However, different effects were obtained when the concentration of cycloheximide varied: no influence was observed with 0.2 mg/ml, but in the presence of 1 mg/ml the basal release and both phases of the A₂ cell response to arginine were completely inhibited (Table 5).

Fig. 3. Influence of vincristine concentration on A₂ cell response to arginine. Control data (not exposed to vincristine) are presented in the left panel ($n = 9$). Infusion of vincristine 2.10^{-6} mmol/l, started 45 min before the stimulation by arginine ($n = 6$), induced a significant increase in first phase ($p < 0.01$) and second phase ($p < 0.001$) of A₂ cell response (central panel). Vincristine 10^{-4} mol/l, also perfused for 45 min ($n = 3$) reduced significantly ($p < 0.05$) both phases of the A₂ cell response to arginine (right panel). Same presentation as in Figure 1

Table 5. Influence, on glucagon release by the isolated perfused rat pancreas of cytochalasin B, of puromycin and cycloheximide

		n	Basal release (ng/min)	Arginine-stimulated release (ng)	
				0–5th min	5–40th min
Controls		9	0.7 ± 0.1	9.4 ± 0.9	59 ± 10
Dimethyl sulfoxide	1/100 (v/v)	4	0.5 ± 0.1	8.6 ± 2.2	60 ± 8
Cytochalasin B	10 μ g/ml	4	2.6 ± 0.4^a	28 ± 3^b	140 ± 20^c
Puromycin	0.1 ng/ml	4	0.7 ± 0.1	5.5 ± 2.5	17.5 ± 0.5^b
Cycloheximide	0.2 mg/ml	5	0.5 ± 0.2	8.0 ± 2	42.2 ± 9.8
	0.5 mg/ml	8	0.5 ± 0.1	6.9 ± 1.2	32.1 ± 5.3^a
	1.0 mg/ml	3	0.1 ± 0.0^b	1.3 ± 1.2^c	7.2 ± 2.4^b

^a $p < 0.05$

^b $p < 0.01$

^c $p < 0.001$

Discussion

The prominent feature demonstrated by the present study is time and concentration dependency for the dual influence of antitubulins on the A₂ cell. For both colchicine and vincristine, the stimulated glucagon release was enhanced by lower concentrations and/or shorter exposures; it was inhibited by higher concentrations and/or longer exposures. The early phase of secretion was more influenced by the enhancing effect; in contrast the inhibition was greater on the second phase.

This dual effect on A₂ cells is very similar to that documented for B cells with colchicine and vincristine [20, 21]. Thus, the marked differences in metabolic control of secretion [28] may not be accompanied by profound differences in the cytophysiology of emiocytosis. This dual effect can account for some of the discrepancies which ap-

peared in previous studies: in a static incubation medium, the glucagon hypersecretion during the very early stages of antitubulin interaction with the A₂ cell may finally result in an increased overall secretion [16].

The present results are consistent with a previous study in which fragments of duct-ligated rat pancreas were incubated in vitro, the incubation medium being changed every 30 min [17]. The inhibitory effect appeared, in the present work, with concentrations of the antitubulins lower, and incubation times shorter than in that study, presumably because drugs reach the islet cells in the perfused pancreas more readily than in incubated fragments.

The reason(s) why the mitotic-spindle inhibitors both facilitate and inhibit the release of insulin and glucagon await(s) further biochemical and ultrastructural studies. Many arguments suggest that the inhibitory effect of these agents on A₂ and B cell

secretions is specifically mediated by the biophysical modifications of microtubules (reviews in 30, 31). A metabolic mechanism occurring inside A₂ cells seems unlikely: the decrease in ATP production documented for high colchicine concentrations [32] could account for glucagon oversecretion [29] and the suppression of insulin release, but not the successive dual effects observed in both types of cells after a graduated exposure; furthermore, no impairment of glucose and octanoate oxidation occurred in such oversecreting islets [16]. An early and transient membrane effect, modifying the membrane/amino-acid interaction, cannot be excluded, since colchicine, to some extent, binds to the cell membranes [33].

The more attractive hypothesis is that hormonal hypersecretion results directly from the early stage of interaction between microtubules and the antitubulin agent; then hyposecretion, in turn, results from a more pronounced interaction. Early paracrystalline deposits have been observed in vinca-alkaloid-treated A₂ [16] and B [21] cells; the disappearance of microtubules was progressive [21]. Similarly colchicine, which prevents the polymerization/depolymerization cycle of tubulin in a way different from vincristine [33, 34] diffuses progressively across the cell membrane [36]; its binding to tubulin is also time-dependent [30–36].

It has been suggested that a functional alteration of the microtubular-microfilamentous system may result in a disoriented intracellular movement of secretory granules [21]. The successive stages of structural alterations observed in vincristine-treated microtubules [37] may provide a morphological basis for the time-dependent appearance of the dual opposite effects: the microtubules first unravel into loosely organized helices; then appear tightly organized helices and finally compact coils [37].

The strong facilitation by cytochalasin B of the arginine-stimulated release suggests a specific action directed towards the cell web. An impairment of glucose uptake, induced by the mould metabolite at the level of A₂ cells [38–40], seems improbable for the concentration of cytochalasin B used, but the persistence of this effect in the presence of slightly higher glucose concentrations [21] was not tested. A more likely explanation for the discrepancies concerning the effects of cytochalasin and colchicine upon the basal glucagon release [14, 15] may be that basal conditions were not actually reached in some of the preparations.

Regarding cycloheximide, a concentration of 0.1 mg/ml should be sufficient to inhibit protein synthesis by 90% at least. Since a concentration of 0.2 mg/ml was ineffective in inhibiting glucagon re-

lease, it is likely that the effects observed with concentration of 0.5 and 1 mg/ml cannot be ascribed to inhibition of protein synthesis, but to other toxic actions [41, 42].

In the absence of concomitant ultrastructural or biochemical studies, the analysis of the modifications induced by pharmacological agents on glucagon secretion can only be hypothetical. It is clear, however, that a limited perturbation of some component of the colchicine-sensitive and vincristine-sensitive system in the A₂ cell favours glucagon release in response to arginine: more profound alterations, obtained by longer incubations and/or higher concentrations, lead to opposite effects. The specificity of antitubulins and other agents as dissecting tools for the secretory process may depend largely upon the experimental conditions.

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