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SHORT COMMUNICATIONS

Effects of cytoskeleton-disrupting drugs on ouabain-stimulated catecholamine secretion from cultured adrenal chromaffin cells

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Since cytoskeleton-disrupting drugs, such as colchicine, vinca alkaloids and cytochalasin B, were first reported to inhibit catecholamine secretion from adrenal medula, it has been thought that cytoskeletons may participate in the secretion as a possible factor regulating the activity of the exocytotic secretory system [1–4]. However, further studies have shown that the inhibition of catecholamine secretion by these cytoskeleton-disrupting drugs is probably due to their direct actions on the plasma membranes, but not based on their disrupting actions on cytoskeletons within the cell, thus providing evidence against a possible involvement of cytoskeletons in the mechanism of stimulus–secretion coupling [5–9]. On the other hand, it has been reported recently that the introduction of DNase I or heavy meromyosin into isolated adrenal chromaffin cells with the aid of liposomes induces depolarization of the plasma membranes and also stimulates the influx of sodium and calcium ions into the cells followed by catecholamine secretion. These findings have led the authors to suggest the possibility that the plasma membrane-associated microfilaments may be involved in a possible mechanism regulating the movement of ions through the plasma membranes, thus influencing the secretory response of the chromaffin cell [10–12]. In view of these findings, it may be fair to say that the conclusive evidence for a possible involvement of cytoskeletons in the secretory process has not yet been obtained.

In the present study, we examined the effects of cytoskeleton-disrupting drugs on the secretory response of the chromaffin cell to different types of secretagogues and found that catecholamine secretion induced by ouabain was enhanced markedly by pretreatment of the cells with cytochalasin B. In contrast, neither colchicine nor vinblastine had any significant influence on the secretory action of ouabain. These results, therefore, seem to indicate that cytochalasin B may cause the enhancement of catecholamine secretion induced by ouabain probably through its disrupting action on microfilaments within the cells, thus providing evidence for a possible involvement of the microfilament system in the mechanism of the secretory action of ouabain on the chromaffin cell.

Materials and methods

Chromaffin cells were enzymatically prepared from fresh bovine adrenal medulla and then cultured for 3 days as described previously [13, 14]. Cells were first preincubated at 37° for 30 min in 250 μl of balanced salt solution [135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose, and 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)/NaOH, pH 7.4] containing various cytoskeleton-disrupting drugs, and then incubated at 37° for different time periods after replacing the preincubation medium with the same volume of balanced salt solution containing 10 μM ouabain without cytoskeleton-disrupting drugs.

Catecholamine secretion observed during the second incubation period was determined as described previously [15], and expressed as a percentage of the total cellular catecholamine content.

The NaCl concentration in the high K^+-medium was reduced to make the solution isotonic. Cytoskeleton-disrupting drugs were stored in a freezer as stock solutions dissolved in dimethyl sulfoxide and then were diluted with balanced salt solution before use.

Colchicine, vinblastine, cytochalasin B, carbamylcholine, and ouabain were obtained from the Sigma Chemical Co. Other chemicals used were of commercially available reagent grade.

Results and discussion

The effects of cytoskelton-disrupting drugs on catecholamine secretion were examined using cultured bovine adrenal chromaffin cells. To avoid the direct interaction of cytoskeleton-disrupting drugs with secretagogues on the surface of the plasma membranes, chromaffin cells were treated with the drugs and then stimulated by secretagogues in the absence of the drugs. As shown in Fig. 1, catecholamine secretion was induced by stimulation of chromaffin cells with 10 μM ouabain, and the ouabain-induced secretion gradually increased according to the incubation time. Pretreatment of the cells with 50 μM cytochalasin B caused not only a slight increase in the basal level but also a further increase in the secretion induced by ouabain. In contrast, neither colchicine nor vinblastine was found to

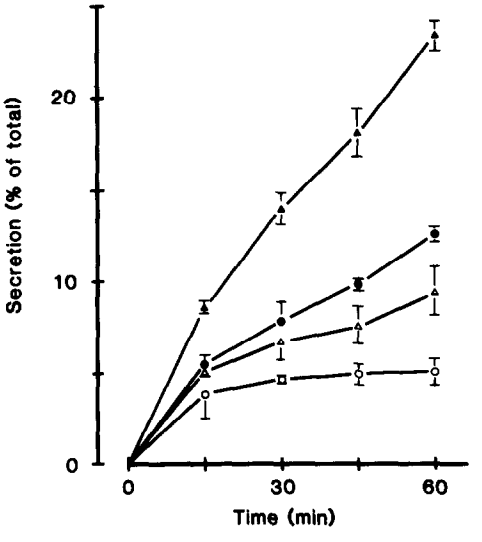

Fig. 1. Effect of cytochalasin B on ouabain-induced catecholamine secretion from cultured adrenal chromaffin cells. Cells were preincubated with (▲, △) or without (●, ○) 50 μM cytochalasin B at 37° for 30 min, and then incubated further at 37° for different time periods in the presence (●, ▲) and absence (○, △) of 10 μM ouabain. Catecholamine secretion was determined as described in the text. Values are the means ± SD of three experiments.

cause any significant change in the ouabain-induced secretion (Fig. 2). These results seem to provide evidence for a possible involvement of microfilaments in the secretory mechanism activated by ouabain. However, it is still questionable whether the enhancement of ouabain-induced catecholamine secretion observed here reflects the disrupting action of cytochalasin B on the microfilament system within the cell.

In view of the previous findings that cytoskeleton-disrupting drugs cause the inhibition of catecholamine secretion induced by acetylcholine or high K^+ probably through their direct actions on the plasma membranes [5–9], it seemed possible that the enhancement of the ouabain-induced secretion by cytochalasin B might be due to its non-specific and toxic action on the cells. Therefore, the effect of cytochalasin B on the secretory response of chromaffin cells to both carbamylcholine and high K^+ was studied again and compared with that on the secretory response to ouabain. As shown in Table 1, although the stimulation of ouabain-induced secretion by cytochalasin B was clearly observed, the secretion induced by 100 μM carbamylcholine of 56 mM KCl was slightly inhibited rather than stimulated by pretreatment of the cells with cytochalasin B under the same conditions. Furthermore, the stimulatory effect of cytochalasin B on the secretion induced by ouabain was dependent on its concentration; this effect was already apparent at 2.5 μM, which may have no influence on the cell viability (Fig. 3). In fact, these disrupting drugs did not cause any significant change in the total cellular catecholamine content, which is considered to indicate approximately the number of the viable cells, at the concentration range used here. (Total catecholamine content within the cells pretreated with a 50 μM concentration of cytochalasin B, vinblastine, colchicine, or without any drug was 113.5 ± 3.4, 120.6 ± 8.3, 107.8 ± 9.7, and 116.6 ± 6.8 nmol/10^6 cells respectively). Therefore, the possibility that the enhancement of ouabain-induced catecholamine secretion by cytochalasin B may be due to its toxic action on the cells seems unlikely.

It is well established that catecholamine secretion induced by carbamylcholine or high K^+ is initiated by a rapid and transient elevation of the free calcium con-

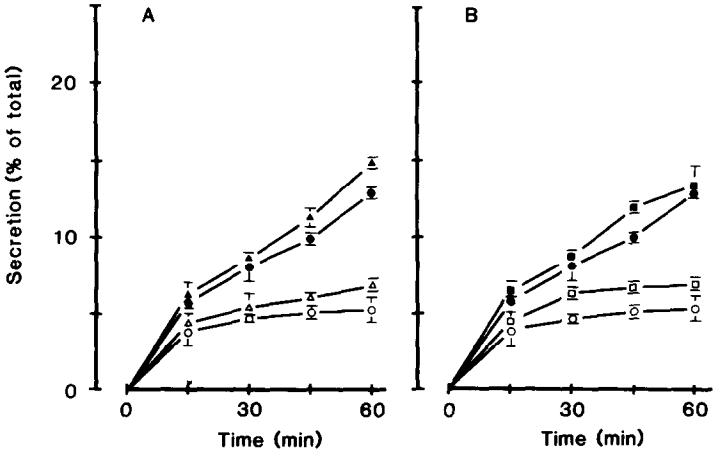

Fig. 2. Effects of colchicine (A) and vinblastine (B) on ouabain-induced catecholamine secretion from cultured adrenal chromaffin cells. Cells were preincubated with 50 μM colchicine (▲, △), vinblastine (■, □) or without the drugs (●, ○) at 37° for 30 min, and incubated further at 37° for different time periods in the presence (closed symbols) and absence (open symbols) of 10 μM ouabain. Catecholamine secretion was determined as described in the text. Values are the means ± SD of three experiments.

Table 1. Effect of cytochalasin B on catecholamine secretion induced by different types of secratogogues from cultured bovine adrenal chromaffin cells

	Catecholamine sectetion induced (%)	
Secretagogue	Control cells	Pretreated cells
Carbamylcholine (100 μM)	6.4 ± 0.2	5.1 ± 0.1
High K^+ (56 mM)	9.7 ± 0.4	7.9 ± 0.2
Ouabain (10 μM)	1.1 ± 0.1	2.3 ± 0.3

Cells were preincubated with 50 μM cytochalasin B at 37° for 30 min and then incubated further at 37° for 15 min in the presence of different secretagogues. Catecholamine secretion was measured as described in the text, and the induced secretion was then obtained by subtracting the value of basal secretion from that of stimulated secretion. The basal secretion values of the control and the pretreated cells were 3.8 ± 0.2 and 4.5 ± 0.5% respectively. Total catecholamine content within the cells without any drug was 116.6 ± 6.8 nmol/10^6 cells.

Values are the means ± SD of three experiments.

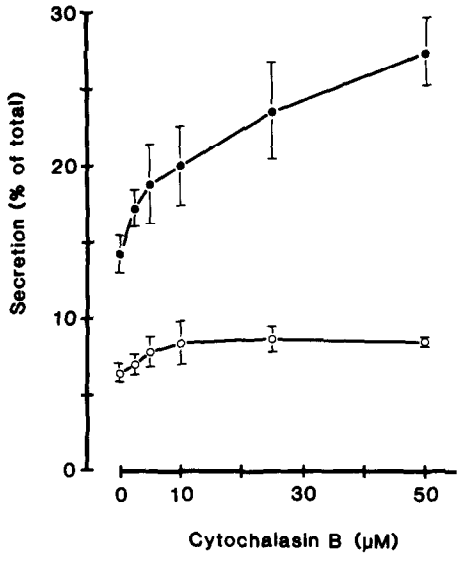

Fig. 3. Effect of the increasing concentration of cytochalasin B on ouabain-induced catecholamine secretion from cultured adrenal chromaffin cells. Cells were preincubated with different concentrations of cytochalasin B at 37° for 30 min, and then incubated at 37° for 60 min in the presence (●) and absence (○) of 10 μM ouabain. Catecholamine secretion was determined as described in the text. Values are the means ± SD of four experiments.

centration in the cell cytoplasm following the influx of calcium ions into the cell through the receptor-mediated or voltage-dependent channels. In contrast, the secretion induced by ouabain is known to be a relatively slow and long-lasting response, but the mechanism of this secretory action is not established yet. As a possible mechanism for the secretory action of ouabain, it has been proposed that the accumulation of sodium ions within the cell resulting from the inhibition of the sodium pump by ouabain may cause the influx of calcium ions into the cell probably through the sodium–calcium exchange mechanism, thus resulting in an elevation of the intracellular free calcium levels followed by the activation of the secretory process [16]. On the other hand, we have shown previously that the interaction of chromaffin granule membranes with F-actin results in the stabilization of ATPase in the membranes *in vitro*, thus suggesting a possible role of the membrane-associated microfilaments as a factor regulating the activity of ATPase in the plasma membranes as well as the granule membranes [17]. In view of these findings, it seems conceivable that the enhancement of the ouabain-induced secretion observed here may reflect an alteration in the sensitivity of the sodium pump to ouabain, which is probably due to the disruption by cytochalasin B of microfilaments associated with the plasma membranes.

In addition to this possible mechanism described above, the possibility that a second mechanism may be involved in the secretory action of ouabain has already been proposed [18]. It has also been reported that catecholamine secretion induced by ouabain may not be accompanied by the influx of calcium ions into the cell [19]. It therefore is still uncertain whether the effect of cytochalasin B on the secretory action of ouabain is due to an alteration in the redistribution of sodium and calcium ions across the plasma membranes as a result of the potentiation of the inhibitory action of ouabain on the sodium pump. However, we have preliminarily examined the uptake of radioactive calcium and rubidium into chromaffin cells and found that the effect of ouabain on the uptake of these ions, as well as that on catecholamine secretion, was potentiated by cytochalasin B.* The results presented here, therefore, seem to provide evidence supporting the possibility that cytochalasin B may cause the stimulation of calcium influx into the cells following the inhibition of the sodium pump by ouabain, thus resulting in the enhancement of the secretion induced by ouabain. Further studies to obtain more direct evidence for the possibility that the effect of cytochalasin B on the secretory action of ouabain may be due to the disruption of microfilaments associated with the plasma membranes are now in progress.

Department of Pharmacology
Tokushima University School of Medicine
Tokushima 770, Japan

KYOJI MORITA†
MOTOO OKA
SHUICHI HAMANO

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† Address correspondence to: Dr. Kyoji Morita, Department of Pharmacology, Tokushima University School of Medicine, 3–18–15 Kuramoto, Tokushima 770, Japan.