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Effects of hypoglycemic sulfonylureas on catecholamine secretion and calcium uptake in cultured bovine adrenal chromaffin cells

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Hypoglycemic sulfonylureas have been widely used in the treatment of adult-onset diabetes mellitus, and these drugs are generally thought to produce their hypoglycemic actions through the direct stimulation of insulin release from pancreatic islets [1]. However, little is known about the details of the mechanism by which sulfonylureas cause their stimulatory actions on insulin release from pancreatic β-cells, although it has been reported that the stimulatory actions of these drugs may require adequate concentrations of extracellular calcium ion [2]. Recent studies have shown that sulfonylureas facilitate the translocation of calcium into an organic immiscible phase or across an artificial lipid membrane [3–6]. Thus, the ionophoretic properties of these drugs have been given considerable attention as a possible mechanism for their stimulatory actions. However, it is still questionable whether the observations obtained from these artificial experimental systems might be relevant in explaining the effects of sulfonylureas on the secretory function of the pancreatic islet. It has been shown recently that the stimulation of insulin release by sulfonylureas is accompanied by calcium influx into pancreatic β-cells through voltage-dependent calcium channels as a result of depolarization of the plasma membrane [7–10]. These observations thus seemed to provide evidence against the possibility that the stimulation of insulin release by sulfonylureas might be due to their ionophoretic actions.

In view of these earlier findings, it may be fair to say that the effects of sulfonylureas on the functions of the plasma membrane of secretory cells have not yet been elucidated. We therefore examined the effects of sulfonylureas on catecholamine secretion and calcium uptake in adrenal chromaffin cells to obtain further information about this question.

Materials and methods

Bovine adrenal chromaffin cells were dispersed enzymatically and cultured for 3 days as described previously [11]. Cells were washed with 1 ml of balanced salt solution consisting of 135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose and 20 mM HEPES*/NaOH (pH 7.4), and then incubated with various secretagogues at 37° for 10 min in 250 μl of balanced salt solution containing different concentrations of sulfonylureas. At the end of the incubation period, the medium was collected and the cells were lysed by adding 250 μl of 10% acetic acid and subjecting them to freeze-thawing. Both medium and lysate were then centrifuged at the maximum speed (approx. 8800 *g*) for 2 min in an Eppendorf centrifuge, and catecholamines in the supernatant fractions were determined as described previously [12]. Catecholamine secretion was routinely expressed as percentage of the total cellular content, which was estimated as approximately 97.1 to 126.4 nmol/10^6 cells (mean ± SE of seven different preparations = 113.5 ± 4.7 nmol/10^6 cells).

In the experiments measuring calcium uptake, cells were washed and incubated with secretagogues at 37° for 10 min in balanced salt solution containing $^{45}CaCl_2$ (3 μCi/ml). At the end of the incubation period, the medium was discarded and the cells were washed three times with 1 ml of ice-cold calcium-free balanced salt solution. The cells were lysed by adding 0.4 N perchloric acid and by subjecting them to freeze-thawing. The radioactivity in the acid extracts was determined by liquid scintillation spectrometry. The amount of calcium taken up into the cells, expressed as nanomoles per 10^6 cells, was calculated on the basis of the specific activity of radioactive calcium in the incubation mixture.

$^{45}CaCl_2$ was obtained from the Amersham Corp. A23187 was purchased from the Aldrich Chemical Co. Hypoglycemic sulfonylureas were donated by Hoechst Japan. The other chemicals used were commercially available reagent grade.

Results and discussion

The influence of sulfonylureas on catecholamine secretion from cultured bovine adrenal chromaffin cells was studied to test the possibility that these drugs might modulate the functions of the plasma membrane, which may be closely related to the secretory mechanism. As shown in Fig. 1, catecholamine secretion evoked by high K^+ was inhibited by both tolbutamide and glibenclamide in a concentration-dependent manner. The inhibitory effect of glibenclamide was more pronounced than that of tolbutamide. In addition, these drugs were shown to inhibit catecholamine secretion evoked by either carbamylcholine or veratridine in a similar manner, but neither tolbutamide nor glibenclamide influenced the basal secretion (data not shown). In contrast, catecholamine secretion induced by the calcium ionophore A23187, which is known to activate the secretory process without stimulating the plasma membrane, was shown to be insensitive to these drugs (Fig. 2). In addition to confirming the earlier observations that

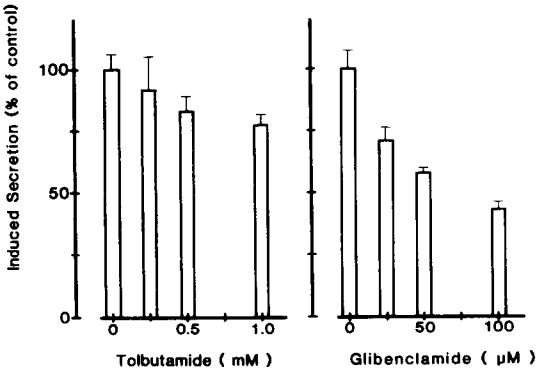

Fig. 1. Effects of hypoglycemic sulfonylureas on high K^+-induced catecholamine secretion from cultured bovine adrenal chromaffin cells. Cells were incubated at 37° for 10 min in high K^+-medium containing various concentrations of sulfonylureas, and catecholamine secretion was determined as described in the text. Results were expressed as a percentage of the control, which was obtained in the absence of the drugs. Values are the mean ± SE of three experiments. A statistically significant decrease in the secretion was observed by tolbutamide at 1 mM and by glibenclamide at 25 μM or higher ($P < 0.005$).

* HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

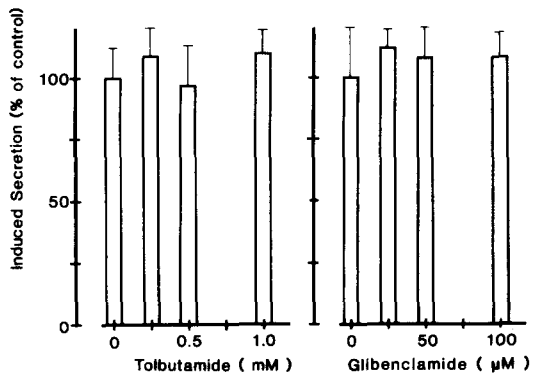

Fig. 2. Effects of hypoglycemic sulfonylureas on A23187-induced catecholamine secretion from cultured bovine adrenal chromaffin cells. Cells were incubated with 10 μM A23187 at 37° for 10 min in the medium containing various concentrations of sulfonylureas. Catecholamines were determined as described in the text. Results were expressed as a percentage of the control, which was obtained in the absence of the drugs. Values are the mean ± SE of three experiments. Statistically significant effects of these drugs on the secretion was not observed at any concentrations used.

hypoglycemic sulfonylureas inhibit catecholamine secretion evoked by stimulation of the acetylcholine receptors from the perfused cat adrenal gland [13], the present results seem to indicate that these hypoglycemic drugs inhibit catecholamine secretion evoked by depolarizing agents through direct actions on the plasma membrane rather than on the intracellular secretory system.

To confirm that the actions of sulfonylureas were exerted on the plasma membrane, the effects of these drugs on calcium uptake into chromaffin cells were examined under the same conditions in which the inhibitory actions of these drugs on catecholamine secretion were observed. As shown in Table 1, glibenclamide inhibited calcium uptake stimulated by high K^+, veratridine, or carbamylcholine at the same concentration that caused the inhibition of catecholamine secretion. There was no significant change observed in basal calcium uptake (data not shown). These results, therefore, seem to indicate that sulfonylureas may act directly on the plasma membrane and inhibit catecholamine secretion as a result of the inhibition of calcium influx into the cells. However, details of the mechanism by which these drugs inhibit calcium influx into chromaffin cells are still unclear. In view of the fact that sulfonylureas stimulate rather than inhibit calcium influx into pancreatic β-cells [7–10], the possibility that the inhibition of calcium uptake by these drugs is due to their non-selective stabilizing actions on the plasma membrane seems unlikely. Thus, sulfonylureas are thought to inhibit calcium influx into chromaffin cells probably through their blocking actions on both receptor-mediated and voltage-dependent calcium channels.

In contrast to the stimulatory effects of sulfonylureas on the pancreatic islet, these drugs cause inhibitory actions on the adrenal medulla. This discrepancy seems to reflect the difference between the pancreatic secretory system and the adrenal secretory system. In the pancreatic secretory process activated by glucose, it has been reported that cationic events are tightly coupled with metabolic events [14]. As a possible explanation for the opposite effects of sulfonylureas on these secretory systems, it seems conceivable that sulfonylureas stimulate calcium influx followed by insulin release, presumably through their primary actions on intracellular metabolism in the pancreatic β-cell, while these drugs inhibit calcium influx, probably through their direct actions on calcium channels in the plasma membrane, and result in the inhibition of catecholamine secretion from adrenal chromaffin cells. However, we have no evidence supporting this idea.

In summary, hypoglycemic sulfonylureas caused a concentration-dependent inhibition of catecholamine secretion evoked by either high K^+ or carbamylcholine, but not by the calcium ionophore A23187 from cultured bovine adrenal chromaffin cells. These drugs also inhibited calcium uptake into the cells induced by both depolarization of the plasma membrane and stimulation of acetylcholine receptors. Thus, the results presented here seem to indicate that sulfonylureas may act directly on the plasma membrane and result in the inhibition of catecholamine secretion through blocking the calcium channels. These observations suggest that an alteration in plasma membrane function may be important as a primary action of sulfonylureas in the adrenal medulla.

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Table 1. Effect of glibenclamide on radioactive calcium uptake into cultured bovine adrenal chromaffin cells

	Stimulated calcium uptake (nmol/10^6 cells)	
Stimulation	None	Glibenclamide
KCl (56 mM)	4.93 ± 0.18	1.10 ± 0.10
Veratridine (20 μM)	1.99 ± 0.08	0.31 ± 0.20
Carbamylcholine (100 μM)	2.67 ± 0.12	0.70 ± 0.11

Cells were incubated with or without 100 μM glibenclamide at 37° for 10 min in balanced salt solution containing secretagogues and $^{45}CaCl_2$ (3 μCi/ml). The cells were washed and lysed as described in the text, and radioactivity in the lysate was then determined. Basal calcium uptake was 1.06 ± 0.12 nmol/10^6 cells. Values are the mean ± SE of three experiments. The decrease in the stimulated calcium uptake caused by glibenclamide was statistically significant ($P < 0.005$).

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