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Effect of hypoglycemic sulfonylurea, glibenclamide, on the rate of catecholamine synthesis in cultured adrenal chromaffin cells

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Hypoglycemic sulfonylureas are generally known to induce their therapeutic actions through direct stimulation of insulin release from pancreatic islets. However, although the stimulatory actions of sulfonylureas on insulin release have already been reported to require adequate concentration of extracellular calcium ions [1], the mechanism of their stimulatory actions on insulin release remains to be elucidated. Recently, hypoglycemic sulfonylureas have been shown to cause the stimulation of insulin release as a result of stimulating calcium influx into pancreatic β-cells probably through voltage-dependent calcium channels [2–5]. On the contrary, we have previously reported that hypoglycemic drugs can inhibit catecholamine release induced by either depolarization of the plasma membrane or stimulation of the acetylcholine receptors from cultured bovine adrenal chromaffin cells, and found that the inhibition of catecholamine release by these drugs is accompanied by the inhibition of calcium influx into the cells as a result of blocking receptor-mediated and voltage-dependent calcium channels [6]. On the other hand, it has already been well established that the stimulation of catecholamine release by various secretagogues is generally accompanied by the stimulation of catecholamine biosynthesis, which is also known to be dependent on the presence of extracellular calcium ions [7]. It therefore seemed reasonable to assume that the drugs affecting catecholamine release might cause some influence on catecholamine biosynthesis in various sympathoadrenergic tissues.

In the present study, the effect of hypoglycemic sulfonylurea, glibenclamide, on catecholamine biosynthesis was then examined in cultured bovine adrenal chromaffin cells, and found that the drug inhibited the formation of [^{14}C]catecholamines from [^{14}C]tyrosine probably through its inhibitory action on calcium influx into the cells.

Materials and methods

Chromaffin cells were enzymatically prepared from fresh bovine adrenal medulla and cultured for 3–4 days as described previously [8]. Cells were washed with 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], and then incubated with or without the hypoglycemic drug at 37° for 60 min in 500 μL of balanced salt solution containing 20 μM [^{14}C]tyrosine (0.25 μCi) with or without stimulants. After removing the incubation medium, the cells were washed twice with 1 mL of ice-cold balanced salt solution, and then lysed by adding 500 μL of 0.4 M perchloric acid. The cell lysate was centrifuged at 9000 *g* for 5 min, and radioactivity in an aliquot (50 μL) of the supernatant fraction was then counted by liquid scintillation spectrometer to determine the amount of [^{14}C]tyrosine taken up into the cells. [^{14}C]Catecholamines contained in the rest of the acid extract (400 μL) were isolated onto aluminum hydroxide gel as reported previously [9], and radioactivity eluted from the gel was then determined by liquid scintillation spectrometry.

Tyrosine hydroxylase was purified from the soluble fraction of bovine adrenal medullary tissues according to the method reported previously [10]. Th enzyme activity was determined by measuring the amount of L-DOPA formed from L-tyrosine during the incubation period. The mixture containing 200 mM Tris/acetate (pH 6.0), 1 mM $FeSO_4$, 100 mM 2-mercaptoethanol, 1 mM 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$), 0.2 mM L-tyrosine, and a suitable amount of the enzyme (about 100–150 μg protein) in a final volume of 500 μL was incubated at 37° for 10 min, and the reaction was stopped by adding 500 μL of 1 M perchloric acid. The amount of L-DOPA formed enzymatically during the incubation period was directly measured by HPLC method as reported previously [11].

L-[U-^{14}C]Tyrosine was obtained from Amersham Japan Corp. (Tokyo, Japan). $DMPH_4$ was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Other chemicals used were commercially available reagent grade. Hypoglycemic sulfonylureas were kindly donated by Hoechst Japan.

Results and discussion

The effect of hypoglycemic sulfonylurea, glibenclamide, on the uptake of tyrosine and the rate of catecholamine biosynthesis was examined in cultured bovine adrenal chromaffin cells. As shown in Table 1, glibenclamide markedly enhanced the basal levels of both [^{14}C]tyrosine uptake and [^{14}C]catecholamine accumulation at the concentration which can produce an inhibitory action on catecholamine release and calcium uptake in adrenal chromaffin cells [6].

Table 1. Effect of glibenclamide on tyrosine uptake and catecholamine biosynthesis in cultured bovine adrenal chromaffin cells

	Tyrosine uptake (pmol/well/hr)	Catecholamine formation (pmol/well/hr)	% Conversion
Basal	730.6 ± 13.0	207.9 ± 15.7	28.5 ± 1.8
+Glibenclamide	1341.9 ± 48.6*	342.6 ± 10.8*	25.5 ± 2.0
High K^+	764.1 ± 14.1	372.1 ± 14.1	48.7 ± 2.4
+Glibenclamide	1196.7 ± 31.6*	368.3 ± 15.3	30.8 ± 5.8*

Cells were incubated with or without 100 μM glibenclamide at 37° for 60 min in the normal and the high K^+ medium containing 20 μM L-[^{14}C]tyrosine. The amounts of [^{14}C]tyrosine and [^{14}C]catecholamines within the cells were determined as described in the text. Values are the mean ± SD of three experiments.

* $P < 0.005$; compared with the values obtained in the absence of the drug.

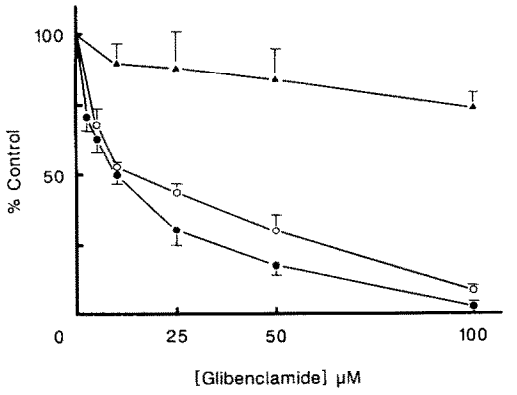

Fig. 1. Effect of glibenclamide on the rate of catecholamine biosynthesis in cultured bovine adrenal chromaffin cells. Cells were incubated with various concentrations of glibenclamide at 37° for 60 min in the medium containing 20 μM L-[^{14}C]tyrosine with 56 mM KCl (●), 300 μM carbamylcholine (○), or 1 mM 8-bromo-cyclic AMP (▲). The amounts of [^{14}C]tyrosine and [^{14}C]catecholamines within the cells were determined, and the conversion rate was calculated as described in the text. Results were expressed as % of control. Values are the mean ± SD of three experiments.

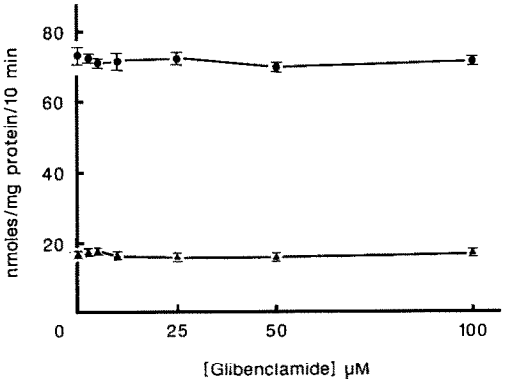

Fig. 2. Effect of glibenclamide on tyrosine hydroxylase prepared from bovine adrenal medulla. The enzyme was incubated with various concentrations of glibenclamide at 37° for 10 min in the reaction mixture containing either saturating [0.2 mM] (●) or subsaturating [0.02 mM] (▲) concentration of L-tyrosine. The amount of L-DOPA formed enzymatically during the incubation period was determined as described in the text. Values are the mean ± SD of three experiments.

These results were considered to indicate that [^{14}C]catecholamine biosynthesis was apparently stimulated by glibenclamide. However, since [^{14}C]tyrosine uptake was thought to exert a critical influence on the formation of [^{14}C]catecholamines probably through an alteration in the precursor supply, it seemed quite reasonable to consider that determination of the amount of [^{14}C]catecholamines accumulated within the cells might not provide the actual rate of catecholamine biosynthesis. Thus, to determine correctly the rate of catecholamine biosynthesis, it seemed necessary to obtain the conversion rate (expressed as the % conversion) of [^{14}C]tyrosine to [^{14}C]catecholamines, which was calculated as the percentage of [^{14}C]tyrosine converted to [^{14}C]catecholamines during the incubation period.

On the basis of the recalculation mentioned above, the results presented in Table 1 clearly indicated that glibenclamide caused no significant influence on the basal rate of catecholamine biosynthesis, which was estimated as an approximate 28% of cellular [^{14}C]tyrosine converted to [^{14}C]catecholamines. In contrast, the rate of [^{14}C]catecholamine biosynthesis was markedly enhanced by stimulation of the cells with high K^+, and the high K^+-stimulated biosynthesis was significantly inhibited by glibenclamide. Thus, glibenclamide was shown to inhibit the stimulatory action of high K^+ on catecholamine biosynthesis in adrenal chromaffin cells.

To characterize further this inhibitory action, the effect of glibenclamide on the stimulatory actions of various stimulants on catecholamine biosynthesis was then examined. As shown in Fig. 1, [^{14}C]catecholamine biosynthesis stimulated by carbamylcholine as well as high K^+ was also markedly inhibited by glibenclamide in a manner dependent on its concentration. In contrast, this drug did not cause any notable inhibitory action on the rate of catecholamine biosynthesis stimulated by either 8-bromo-cyclic AMP (Fig. 1) or phorbol ester TPA (data not shown). In addition, the direct action of glibenclamide on the activity of tyrosine hydroxylase, which is well known as the rate-limiting enzyme in the pathway of catecholamine biosynthesis, was examined *in vitro*, and found that the enzyme activity was not altered by glibenclamide at the concentrations used here in the presence of both saturating and subsaturating concentrations of L-tyrosine (Fig. 2). This drug also failed to affect the enzyme activity even in the presence of subsaturating concentrations of the cofactor (data not shown). These findings seem to indicate that glibenclamide may inhibit the rate of catecholamine biosynthesis as a consequence of inhibiting the stimulatory actions of various secretagogues on the cell surface rather than the intracellular mechanism of catecholamine biosynthesis.

In view of the earlier findings that the stimulatory action of high K^+ or carbamylcholine on both catecholamine biosynthesis and release is mediated by the stimulation of calcium influx into the cells, whereas the stimulatory effect of cyclic AMP on catecholamine biosynthesis is independent of the presence of calcium ions in the extracellular space [7], it therefore seems reasonable to conclude that glibenclamide inhibits catecholamine biosynthesis, which is always stimulated by splanchnic nerve *in vivo*, probably through its blocking action on calcium influx into the chromaffin cell, as previously reported [6].

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Actions of morphine on histamine dynamics in the mouse brain: a strain comparison

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The existence of brain histamine (HA) in both neurons and mast cells within the central nervous system is now well established [1–3]. Brain HA is synthesized by histidine decarboxylation [4] and methylated by HA methyltransferase (HMT) [5], producing *tele*-methylhistamine (t-MH), a substrate for monoamine oxidase (MAO) [6, 7]. After irreversible inhibition of MAO by pargyline, the rate of accumulation of t-MH has been used to estimate brain HA turnover rate [8–11], a parameter thought to be an index of histaminergic neuronal activity under basal conditions [12].

Recent studies suggest that brain HA may mediate some central actions of morphine (MOR). For example, a series of brain-penetrating H_2 antagonists inhibited MOR antinociception with a potency that paralleled their affinity at the H_2-receptor [13]. Although acute MOR has no effect on the brain levels of HA and t-MH [14–16], Nishibori *et al.* [16] found that MOR increases HA turnover in the ddY mouse brain, consistent with a MOR-induced release of HA. Further results suggest that this effect occurs by activation of *mu* opiate receptors and facilitation of HA release from nerve endings [17]. To compare specific opiate responses to opiate-induced changes in brain HA metabolism, we have presently studied the effect of MOR on brain HA dynamics in three strains of mice known to vary in their responses to opiates [18, 19].

Male mice [Swiss–Webster (SW), C57/BL6 (C57) and DBA/2J (DBA), Taconic Farms, Germantown, NY] weighing between 20 and 30 g were housed in groups of five to six animals per cage in 12-hr light–dark cycles with food and water freely available. Three to four hours into the light cycle of the animals, groups of four to eight mice received pargyline hydrochloride (65 mg/kg, i.p., 10 mL/kg or saline vehicle), immediately followed by a specified dose of MOR sulfate (s.c. or saline vehicle), and were killed by decapitation at the specified intervals. Whole brains were removed and homogenized in 5 vol. of ice-cold deionized water with aliquots (0.1 mL and 0.35 mL) taken for HA and t-MH analysis respectively. Samples were analyzed for HA by the single isotope radioenzymatic assay, and for t-MH by automated gas chromatography–mass spectrometry as previously described [20]. Separate brains were assayed for HMT activity [20].

Table 1 summarizes the baseline value for brain levels of HA, t-MH, HMT activity, and HA turnover rates in three strains of mice. Surprisingly, C57 and DBA whole brain HA

Table 1. Whole brain levels of HA, its metabolite t-MH, HMT activity, and estimated basal HA turnover rates in three strains of mice

Strain	HA (ng/g)	t-MH (ng/g)	HMT activity (μmol/g/hr)	HA turnover rate (pmol/g/min)
SW	56.8 ± 4.4 (8)	127.6 ± 11.3 (6)	1.30 ± 0.02 (4)	5.7 ± 0.7 (12)
C57	83.7 ± 6.2* (11)	171.9 ± 7.4† (14)	1.44 ± 0.04‡ (3)	5.2 ± 0.9 (14)
DBA	88.8 ± 3.1† (25)	143.1 ± 3.3 (25)	1.49 ± 0.02* (3)	8.8 ± 1.0‡ (7)

Animals received pargyline (65 mg/kg, or saline, i.p.) and were decapitated 60 min later. HA turnover rates were determined as molar t-MH levels in the presence of pargyline minus mean control t-MH levels in the absence of pargyline, corrected for treatment time (60 min). Values are means ± SE; the sample size is given in parentheses.

*–‡ Significantly different from SW values: * $P < 0.01$, † $P < 0.005$, and ‡ $P < 0.05$.