Tolbutamide and Glyburide Differ in Effectiveness to Displace α- and β-Adrenergic Radioligands in Pancreatic Islet Cells and Membranes

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SUMMARY

Previous in vivo findings indicated that α-adrenergic blocking agents enhanced tolbutamide-induced insulin secretion, whereas β-blockade attenuated it. In the present study, the interaction of tolbutamide and glyburide with the rat islet adrenergic receptors is examined directly by determining the effectiveness of these drugs to displace the specific α- and β-adrenergic radioligands, [^{3}H]-clonidine and [^{3}H]-dihydroalprenolol (DHA). It was found that both tolbutamide and glyburide had affinity constants for the adrenergic receptors that were similar to those for the natural receptor ligands and powerful antagonists. Tolbutamide displaced both α- and β-radioligands but had a higher affinity at the β-receptor. Glyburide also displaced radioligands from both types of receptors but had a higher affinity for the α-receptor. This study suggests that these two sulfonylurea hypoglycemic agents may affect insulin secretion by different mechanisms.

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The mechanism by which the oral hypoglycemic sulfonylurea drugs lower blood glucose is unknown and continues to be a subject of much interest. One well-established acute effect of these drugs is to increase insulin secretion. Evidence obtained in the dog indicated that stimulation of insulin secretion by tolbutamide can be inhibited by the β-adrenergic blocker propranolol, whereas it was greatly enhanced by α-blockade with phentolamine.[1] These observations suggest that stimulation of insulin secretion by tolbutamide may involve either a direct effect of the drug on islet adrenergic receptors or some indirect consequence of adrenergic receptor activation.

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We have examined the possibility of the direct effects of the sulfonylurea drugs on the pancreatic islet adrenergic receptors by using these drugs to displace specific α- and β-adrenergic radioligands from isolated rat pancreatic islet cells and membranes. The effects of glyburide as well as tolbutamide were studied, since the former is more potent in stimulating insulin secretion. The data reveal that both drugs displace the adrenergic radioligands; tolbutamide has a greater affinity for the β-receptor, whereas glyburide is more effective at the α-receptor.

MATERIALS AND METHODS

Male Sprague-Dawley rats (225–300 g), maintained on lab chow and tap water, were anesthetized with pentobarbital (Nembutal, 40 mg/kg i.p.) and the islets were isolated by the sedimentation method of Lacy and Kostianovsky.[2] The islets were cultured for 24–48 h in RMPI 1640 medium with Earle's Salt (Flow Laboratories, McLean, Virginia) containing 10% newborn calf serum, 2 mg/ml cefazolin, and 1 mg/ml amikacin, and subsequently cells were obtained as previously described.[3] The protein content of the cell suspension was determined by the method of Lowry et al.[4] and ranged from 0.5 to 1.0 mg/ml. Cell viability was determined by the trypan blue dye exclusion test[5] and only material in which at least 95% of the cells were viable was used.

Membranes were obtained from the cultured pancreatic islets by sedimenting the islets and washing five times in a modified Ringers solution[6] containing 100 mg/dl glucose. The islets were then suspended to 10 × volume with modified Ringers and were homogenized with five strokes of a teflon pestle at 500 rpm. The resulting homogenate was centrifuged at 2000 × *g* for 10 min to remove large cell debris. The supernatant was then centrifuged at 20,000 × *g* for 20 min to sediment membrane fragments. The resulting pellet was resuspended in an appropriate volume of assay buffer, usually between 25 and 50 ml.

The binding of tritiated adrenergic ligands to the islet cells

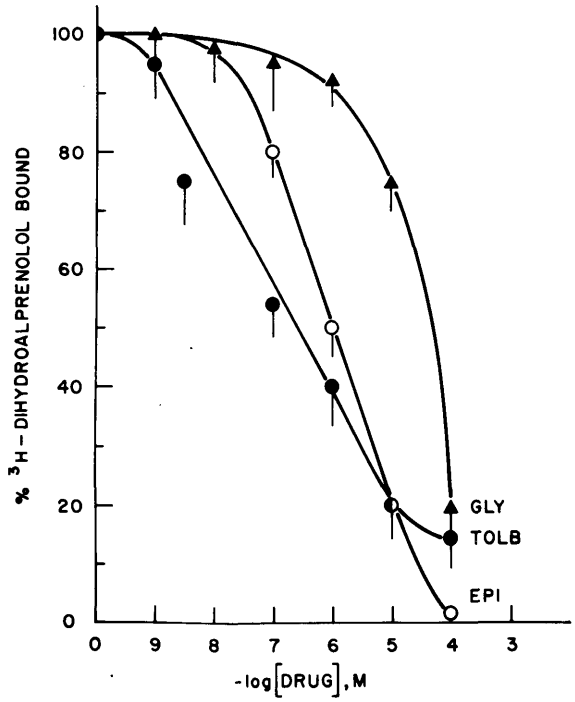

FIGURE 1. Inhibition of binding of the β-adrenergic radioligand [³H]-dihydroalprenolol to islet cells by increasing concentrations of tolbutamide (●), epinephrine (○), and glyburide (▲). Tolbutamide was most effective at inhibiting binding with 50% inhibition (IC_{50}) occurring at 10^{-7} M, yielding an affinity (K_i) of 9.2 ± 0.6 nM.

and membranes was determined using a filter binding assay previously described.[3] [³H]-Clonidine, specific activity 24 Ci/mmol, and [³H]-dihydroalprenolol (DHA) (New England Nuclear, Boston, Massachusetts), specific activity 49 Ci/mmol, were used as the α- and β-receptor ligands, respectively. Incubations were performed in 1.0-ml volumes containing 0.5 ml of cell suspension, 0.2 ml [³H]-ligand, and 0.3 ml 50 mM Tris-HCl/10 mM MgCl (pH 7.4), 100 mg/dl glucose, and the appropriate displacement ligand. Final concentration of [³H]-ligand was 10–20 nM. Incubations were at room temperature (24°C), in triplicate.

In each experiment, nonspecific binding of the [³H]-ligand to the cells and/or filters was determined by measuring the binding in the presence of 10^{-6} and 10^{-5} M phentolamine or 10^{-6} and 10^{-5} M propranolol, for the α- and β-receptors, respectively, and the nonspecifically bound counts were subtracted from the total bound radioactivity to determine the specific binding.

Epinephrine and the sulfonylurea drugs tolbutamide and glyburide were prepared to give final concentrations of 10^{-9} to 10^{-3} M. The displacement ligand was incubated with the radioligand and cells for 1 h and processed as described above. In some experiments, shorter incubations (½ h) were performed with no difference in results.

The results of each displacement were recorded as graphs, and the concentration of drug displacing 50% of the specifically bound ligand, the IC_{50} or $D_{0.5}$, was determined. From these data, the apparent dissociation constant, K_i, of the displacing ligand was determined according to the following calculation:[7]

$$K_i = \frac{IC_{50}}{1 + \frac{[^3H]\text{-ligand}}{K_d}}$$

RESULTS

The binding of the tritiated radioligands [³H]-clonidine and [³H]-DHA to the membrane fragments of pancreatic islets was determined and subjected to a Scatchard analysis.[8] [³H]-Clonidine was found to have an affinity constant (K_d) of 0.38 ± 0.05 nM for the α_2-binding sites of this preparation while the K_d for [³H]-DHA was 0.23 ± 0.06 nM for the β-receptors. The ratio of α- to β-receptors was found to be 1:3.5 ± 0.1 based on the B_{max} calculated by the Scatchard analysis. The K_d values obtained previously[9] for the whole cell preparation were 0.55 nM and 1.23 nM for the α_2- and β-binding, respectively. The ratio of α_2- to β-binding in the whole cells was 1:3.3 ± 0.2. Thus, both whole cells and membrane preparations show the same α- to β-receptor ratio.

Figure 1 shows data on the inhibition of binding of [³H]-DHA to the whole islet cells by epinephrine, tolbutamide, and glyburide. Tolbutamide was the most effective, with 50% inhibition occurring at 3×10^{-7} M. From these data and previously obtained values for the K_d of DHA for the islet cell

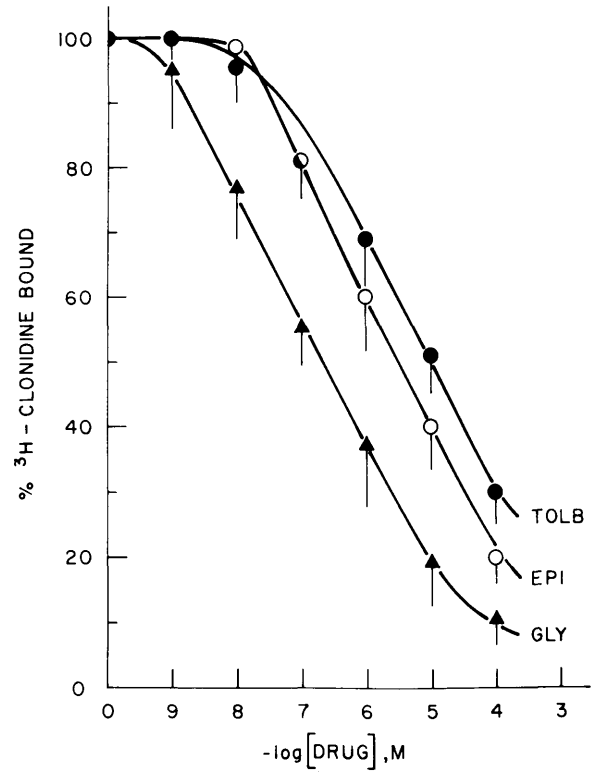

FIGURE 2. Inhibition of binding of the α_2-adrenergic radioligand [³H]-clonidine to islet cells by increasing concentrations of glyburide (▲), epinephrine (○), and tolbutamide (●). The calculated K_i of glyburide for the α_2 receptor is 4.3 ± 0.2 nM.

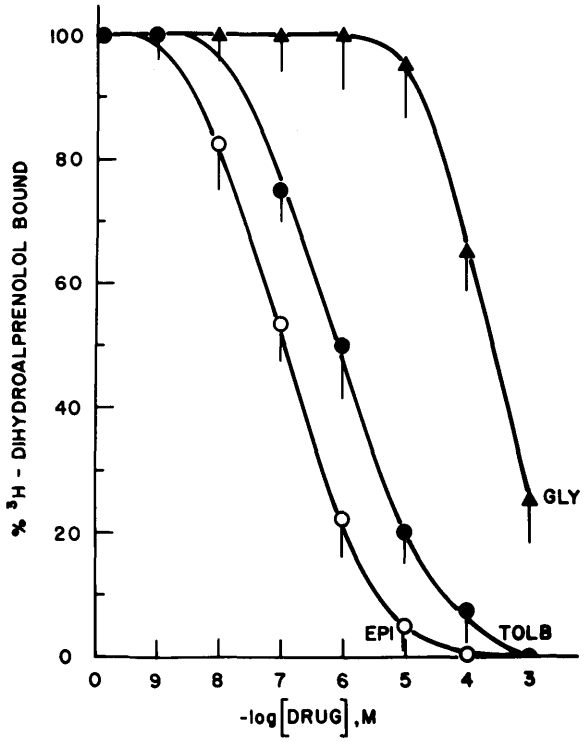

FIGURE 3. Inhibition of binding of [³H]-dihydroalprenolol to β-adrenergic sites of pancreatic islet membrane preparation by epinephrine (○), tolbutamide (●), and glyburide (▲). Epinephrine is most potent in this preparation. Tolbutamide also exhibited a high affinity for these binding sites with a K_i of 22 ± 3 nM.

β-receptor,[3,9] the dissociation constant, K_i, is calculated to be 9.2 ± 0.6 nM. Epinephrine inhibited 50% of the radioligand binding at a concentration of 10^{-6} M, yielding a K_i of 334 ± 20 nM. Glyburide inhibited 50% of [³H]-DHA binding at 2.5 × 10^{-4} M, with a K_i of 55,000 ± 1500 nM.

Figure 2 presents data on inhibition of binding of [³H]-clonidine to the α_2-adrenergic sites of the islet cells. Glyburide was most potent, inhibiting 50% of the radioligand binding at a concentration of 2 × 10^{-7} M, with a K_i of 4.3 ± 0.2 nM. Epinephrine inhibited 50% of the radioligand binding at 2 × 10^{-6} M, yielding a K_i of 150 ± 15 nM. Tolbutamide was least effective, with 50% inhibition occurring at 3 × 10^{-5} M, with a K_i of 8300 ± 350 nM.

Figure 3 shows the curves obtained for inhibition of [³H]-DHA binding to islet cell membranes by epinephrine, tolbutamide, and glyburide. Epinephrine caused a 50% inhibition of binding of the radioligand at a concentration of 10^{-7} M, with a K_i of 3.3 ± 0.6 nM. Tolbutamide inhibited 50% of binding at 10^{-6} M, yielding a K_i of 22 ± 3 nM. Glyburide was least effective in inhibiting [³H]-DHA binding, with 50% inhibition occurring at 5 × 10^{-4} M, giving a K_i of 11,000 ± 500 nM.

Figure 4 presents the data for the inhibition of [³H]-clonidine binding to the islet cell membranes. Glyburide produced the greatest inhibition of the binding membranes, epinephrine was less effective, and tolbutamide was least effective. The 50% inhibition of the radioligand binding for these three agents occurred at, respectively, 8 × 10^{-8} M with K_i 3.4 ± 0.2 nM, 1.5 × 10^{-7} M with K_i 9.1 ± 1.1 nM, and 8 × 10^{-4} M with K_i 36,000 ± 2200 nM. The above data are summarized in Table 1.

Figure 5 shows the Hofstee plots obtained for the binding data shown in Figures 1–4. The plots obtained for epinephrine inhibition of [³H]-DHA (panel A) and [³H]-clonidine (panel C) binding to whole cells show nonhomogeneous competition patterns. The Hill coefficients for these curves were found to be below one. A possible explanation for these nonlinear Hofstee plots, namely, the presence of two states of the receptor for the agonist, was tested by computer analysis of the competition curves using nonlinear least squares curve fitting[10] and evaluating the fit via the residual of the sum of the squares[11] and comparing a model of two independent sites for agonist binding with one-site model. Since the comparison did not yield statistically significant differences ($P > 0.05$), it is unlikely that two receptor sites are involved to give the nonclassical competition curves obtained here. In the membrane fractions, the epinephrine competition data produced linear Hofstee plots (Figure 5, panels B and D) and the Hill coefficients increased to unity.

The drugs glyburide and tolbutamide yielded linear Hofstee plots in both cells and membranes. The Hill coefficient for tolbutamide was unity in all cases, while the Hill coefficients for glyburide were less than unity for competition with [³H]-DHA, and unity for competition with [³H]-clonidine.

DISCUSSION

The present studies were carried out using whole cells, as well as the commonly used membrane preparation. The

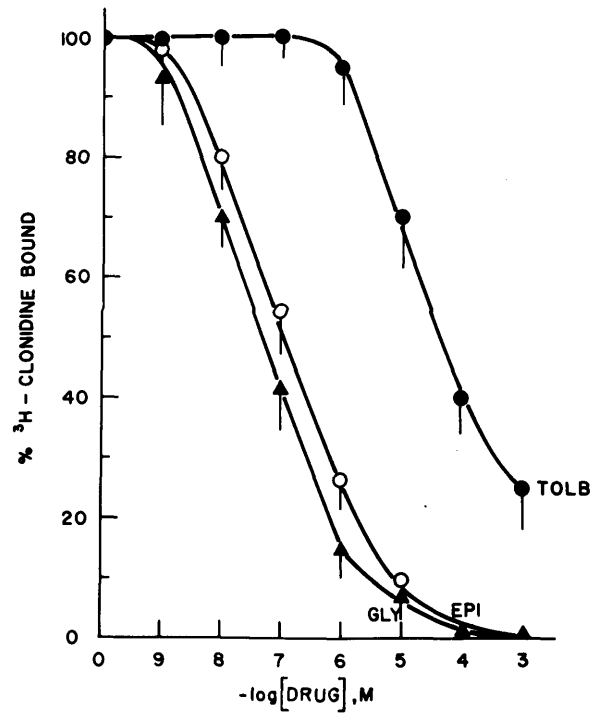

FIGURE 4. Inhibition of the binding of the α_2-radioligand [³H]-clonidine to the membrane preparation by increasing concentrations of glyburide (▲), epinephrine (○), and tolbutamide (●). Glyburide exhibited the highest affinity with a K_i of 3.4 ± 0.2 nM.

TABLE 1
Inhibition of binding of radioligands to cells and membranes by epinephrine, tolbutamide, and glyburide*

	Cells (K_i nM)		Membranes (K_i nM)	
	[^{3}H]-Clonidine	[^{3}H]-DHA†	[^{3}H]-Clonidine	[^{3}H]-DHA
Epinephrine	150 ± 15‡	334 ± 20	9.1 ± 1.1	3.3 ± 0.6
Glyburide	4.3 ± 0.2§	55,000 ± 1,500	3.4 ± 0.2§	11,000 ± 500
Tolbutamide	8300 ± 350§	9.2 ± 0.6	36,000 ± 2200§	22 ± 3

*K_i determined from IC_{50} as described in text; †[^{3}H]-dihydroalprenolol; ‡results are mean ± SEM of six triplicate determinations; §all differences (α versus β, tolbutamide versus glyburide) are statistically significant, $P < 0.001$.

whole cells were used because it is more likely that their membrane receptors would be in a more physiologic state than those in isolated membranes and thus allow a more meaningful appraisal of ligand binding and competition at these receptor sites. A potential complication in using whole cells is the extent of uptake of the radioligands into the cells. Such uptake was minimal here but may account for the higher K_d for DHA obtained with the whole cells compared with the membranes. There may also be degradation of the agonists when using whole cells but this did not occur here probably because varying the length of preincubation with the unlabeled drugs did not affect the data of the competition experiments.

The present results, using cells or membranes, indicate that [^{3}H]-DHA and [^{3}H]-clonidine bind with high affinity to independent, one-state sites. The binding sites are homogeneous toward the adrenergic agonist epinephrine and toward the two sulfonylurea drugs, as indicated by the Hill coefficients of their competition curves. The binding sites on the pancreatic islet cells have previously been shown to be of the α_2- and β_2-type.[3,9]

The major differences in the binding characteristics between the whole cell and membrane were seen with epinephrine, which was approximately 100-fold more potent against [^{3}H]-DHA binding in membranes, and approximately 20-fold more potent against [^{3}H]-clonidine binding in membranes. On the membranes, the competition curves showed Michaelis-Menton behavior, linear Hofstee plots, and Hill coefficients approaching unity, indicating a single-affinity state of the receptors. In contrast, the whole cells exhibited shallow competition curves with Hill coefficients less than one. These findings are essentially the same as those found in other comparisons of the adrenergic receptors on whole cells versus membrane fragments.[12-15] Two other studies have also reported Hill coefficients of less than one for agonist binding to the α[16] and β[14] -adrenergic receptors. The competition curves appear to be shallower when using whole cells in contrast to cell membranes, but the reason for this is not known. It has been suggested that this might be linked to the physiologic actions of the drugs used,[16] but this observation is poorly understood.

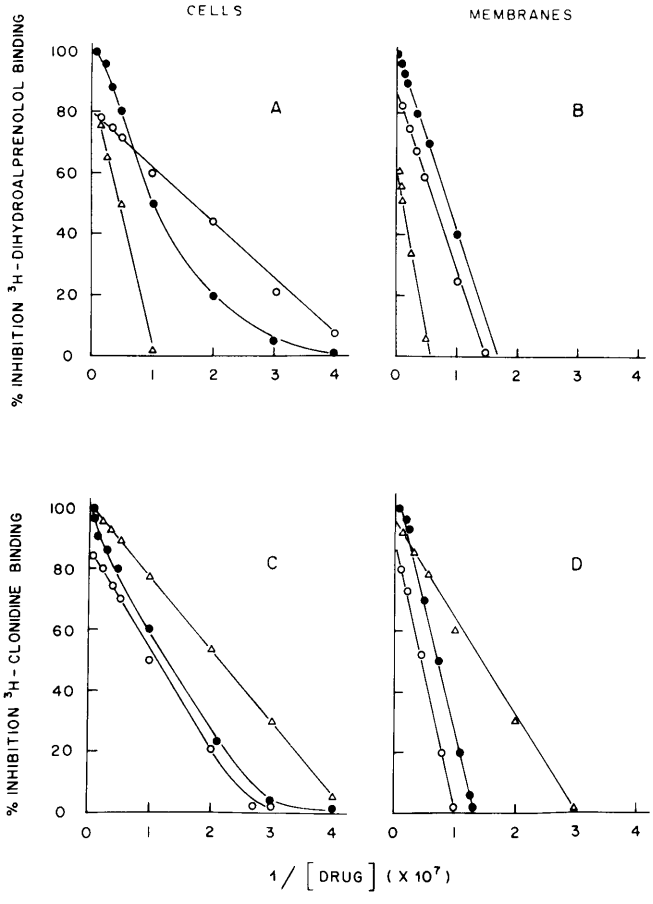

FIGURE 5. Hofstee plots from the specific inhibition of [^{3}H]-dihydroalprenolol binding and [^{3}H]-clonidine binding by glyburide (△), epinephrine (●), and tolbutamide (○), to both intact and broken-cell preparations. Data are taken from Figures 1–4.

The present studies clearly show that the hypoglycemic sulfonylurea drugs have the potential to act at the adrenergic binding sites on the pancreatic islet cells. The biologic significance of these findings must await a better understanding of the role of α- and β-adrenergic receptors in moderating insulin secretion. It is useful, however, to attempt to integrate the in vitro and in vivo data. It is generally accepted that stimulation of α-adrenergic receptors on the pancreatic islets inhibits insulin secretion, whereas stimulation of β-receptors increases it. Furthermore, we have shown earlier that β-receptors outnumber the α-receptors by 3.3 to 1.[9] If these opposing influences are fully operational in influencing insulin secretion, this ratio would suggest that the inhibitory effect of the α-receptors is more potent than the stimulatory effect of the β-receptors. Tolbutamide was found to bind with both α- and β-adrenergic receptors but has a higher affinity for the β-receptor. If we assume that tolbutamide is stimulating insulin release through its action on the β-receptor, then the total insulin response would be attenuated by a concomitant stimulation of the α-receptor. Removal of the α-stimulation by phentolamine would enhance the tolbutamide effect on insulin secretion, and this has been observed in

vivo.[1] Glyburide likewise binds with both the α- and β-adrenergic receptors but has a higher affinity for the α-receptor. Stimulation of the latter should attenuate insulin secretion, whereas it is well documented that glyburide is more potent than tolbutamide in stimulating insulin secretion. This suggests that glyburide may act to stimulate insulin release in a manner somewhat different from that used by tolbutamide. Identification of specific mechanisms must await a better understanding of how the two types of adrenergic receptors affect insulin secretion. The preceding interpretations do not exclude other possible explanations for the greater potency of glyburide to increase insulin secretion, e.g., that glyburide binds more avidly than tolbutamide to islet cell membranes[17] and that the more lipophilic structure of glyburide enhances its penetration into the cells. Nevertheless, the present findings add to a growing number of observations from experimental[18] and clinical[19–22] studies that indicate an involvement of the adrenergic receptors on pancreatic islets in influencing insulin secretion. The present study, using specific adrenergic receptor radioligands, implicates these receptors in the action of sulfonylurea hypoglycemic drugs.

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