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Demonstration of α_2-Adrenergic Receptors in Rat Pancreatic Islets Using Radioligand Binding[1] (41498)

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Abstract. The type of the α-adrenergic receptors on rat pancreatic islet cells was characterized directly using specific radioligands and displacement agonists and antagonists. Scatchard plots for binding of [^{3}H]clonidine (α_2-agonist) revealed a dissociation constant, K_d of 0.552 ± 0.1 n*M* and density of binding sites (B_{max}) of 50.4 ± 3.6 fmole/mg protein. Similar values were obtained with [^{3}H]dihydroergocryptine (antagonist). The various agonists displaced [^{3}H]clonidine with the following order of potency: clonidine > epinephrine ≈ norepinephrine > isoproterenol. Yohimbine, the α_2-antagonist, was very effective in displacing [^{3}H]clonidine, whereas the α_1-antagonist, prazosin, was much less effective. The data indicate that the α-adrenergic receptors on rat pancreatic islets are of the α_2 subtype.

We have previously reported (1) that rat pancreatic islet cells bound [^{3}H]dihydroergocryptine and [^{3}H]dihydroalprenolol, the commonly used α- and β-adrenergic radioligands. Subsequently we have validated the use of these ligands to characterize the presence of α- and β-receptors on islet cells by using a number of displacement ligands (2). The recent availability of ligands and antagonists which act specifically at α_1 and α_2-adrenergic receptors makes it possible to characterize further the type of α-adrenergic receptor on the islet cells. The adrenergic receptors were found to be of the α_2 subtype.

Materials and Methods. Male Sprague–Dawley rats (225–300 g), fed *ad libitum* lab chow and tap water, were anesthetized with pentobarbital (Nembutal, 40 mg/kg, ip) and the islets were isolated by the sedimentation method of Lacy and Kostianovsky (3). They were subsequently resuspended in 25 ml RPMI 1640 (Flow Laboratories, McLean, Va.) containing 10% fetal calf serum, 400 U/ml penicillin, and 200 μg streptomycin, placed in a plastic culture flask, and incubated for 2–5 days at 37° in an atmosphere of 95% O_2/5% CO_2.

Islet cells were obtained by transferring the culture into Ca^{2+} free Krebs bicarbonate solution containing 100 mg/dl glucose and 15 m*M* EGTA and shaking gently for 5 min. The resulting single cell suspension was then centrifuged at 700*g* for 8 min at 4°. For radiobinding assay the cell pellet was resuspended in 50 m*M* Tris–HCl (pH 7.4), containing 10 m*M* $MgCl_2$ and 100 mg/dl glucose. The protein content of this suspension was determined by the method of Lowry *et al.* and ranged from 0.5 to 1.0 mg/ml. Cell viability was determined by the trypan blue dye exclusion test and only material in which at least 95% of the cells were viable was used.

The binding of tritiated drugs to the islet cells was determined using a filter binding method previously described (4). [^{3}H]Dihydroergocryptine, specific activity 27 Ci/mmole and [^{3}H]clonidine, 23 Ci/mmol (Amersham) were used as the α and α_2 ligands, respectively. Incubations were performed in 1.0-ml volumes containing 0.5 ml of cell suspension, 0.2 ml ^{3}H-ligand, and 0.3 ml 50 m*M* Tris–HCl/10 m*M* Mg Cl_2 (pH 7.4), and containing glucose at 100 mg/dl.

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Final concentrations of the appropriate ^{3}H-ligand ranged from 1×10^{-10} to 2×10^{-8} *M*. Incubations were at room temperature (24°), in triplicate.

In each experiment, nonspecific binding of the ^{3}H-ligand to the cells and/or filters was determined by measuring the binding in the presence of a displacing ligand and constituted about 25% of total binding. [^{3}H]Dihydroergocryptine and [^{3}H]clonidine were displaced with 10^{-5} *M* phentolamine and the nonspecifically bound counts were subtracted from the total bound radioactivity to determine the specific binding.

The data from the binding studies were plotted according to the method of Scatchard (5). The dissociation constant (K_d) was calculated as the reciprocal of the slope of the Scatchard plot data and the maximal number of binding sites (B_{max}) from a regression analysis of the Scatchard plot to the abscissa intercept using a linear least-squares program on a Sol Computer.

The displacement radioligands: epinephrine, norepinephrine, isoproterenol, clonidine, prazosin, and yohombine, were prepared to give final concentrations 10^{-9} to 10^{-3} *M* in buffer containing sodium metabisulfite (0.1%) to prevent oxidation. The displacement ligand was incubated with the radioligand and cells for one hour and then processed as described above.

The results of each displacement were recorded on 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 for the binding site was determined according to (6):

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

Results and Discussion. Figure 1 shows the Scatchard plot obtained for the binding of [^{3}H]clonidine. The dissociation constant, K_d, from these data is 0.552 ± 0.1 n*M* and the density of binding sites (B_{max}) was found to be 50.4 ± 3.5 fmole/mg protein.

Data on the displacement of [^{3}H]clonidine (10 n*M*) by adrenergic agonists are shown in Fig. 2. The order of potency was found to be clonidine > epinephrine ≃ norepinephrine > isoproterenol. These data are summarized in Table I. The displacement of [^{3}H]clonidine by the α_1-antagonist, prazosin, compared to that of the α_2-antagonist, yohimbine, is depicted in Fig. 3. The displacement constant, K_i, for yohimbine was found to be 4.25 ± 0.9 n*M* while that for prazosin was 3942 ± 50 n*M*. Similar experiments for the displacement of the nonselective ligand, [^{3}H]dihydroergocryptine, yielded the curves depicted in

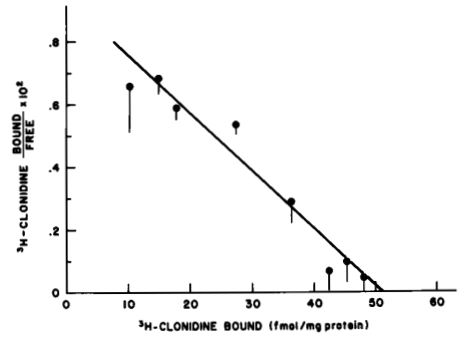

FIG. 1. Scatchard plot of the specific binding of [^{3}H]clonidine to pancreatic islet cells. The K_d for binding is 0.55 n*M* with a B_{max} of 50.4 fmole/mg protein indicating that the binding is α_2.

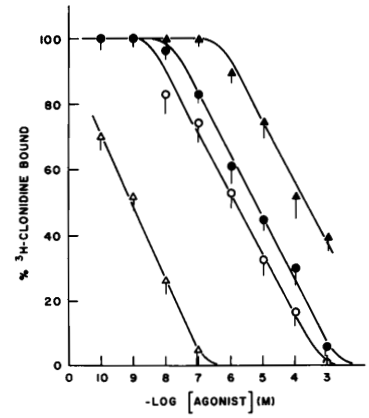

FIG. 2. Displacement of the α_2 radioligand, [^{3}H]clonidine (10 n*M*) by various concentrations of adrenergic agonists, listed in decreasing order of displacement potency: △ Clonidine > ○ epinephrine ≃ ● norepinephrine > ▲ isoproterenol.

TABLE I. BINDING AND DISPLACEMENT OF α- AND α_2-ADRENERGIC RADIOLIGANDS IN RAT PANCREATIC ISLET CELLS

Ligand	B_{max} (fmol/mg protein)	K_d (nM)	K_i (μM) Displacement by			
			Epinephrine (n = 6)	Norepinephrine (n = 6)	Isoproterenol (n = 6)	Clonidine (n = 6)
α_2-Clonidine (5)	50.4 ± 3.6[a]	0.552 ± 0.09	0.150 ± 0.008	0.420 ± 0.013	16.0 ± 1.7	0.523 ± 0.10 nM
α-Dihydroergocryptine (5)	55.0 ± 1.1	0.325 ± 0.11	0.183 ± 0.016	0.183 ± 0.016	9.9 ± 2.9	0.488 ± 0.09 nM

[a] Results are means + SEM. Determination of B_{max} and K_d was done on five animals, K_i on six animals. There is no significant difference between epinephrine and norepinephrine in their ability to displace the α and α_2 radioligands. Both radioligands are displaced most effectively by clonidine.

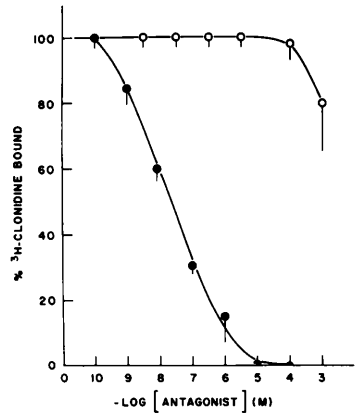

FIG. 3. Displacement curves for yohimbine (●) and prazosin (○) vs 10 nM [^{3}H]clonidine; yohimbine (α_2-antagonist) was found to displace the bound clonidine with an affinity (K_i) similar to that for displacing [^{3}H]dihydroergocryptine. Prazosin (α_1-antagonist) was ineffective suggesting that α-receptors on the pancreatic islet cells are of the α_2 type.

Fig. 4. The K_i for yohimbine was 7.1 ± 75 nM, again indicating the preferential displacement at the α_2-adrenergic receptor.

The use of adrenergic receptor agonists and antagonists *in vivo* and *in vitro* has led to the generally accepted working hypothesis that α-adrenergic receptors on

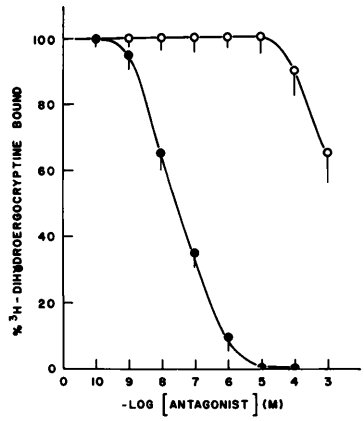

FIG. 4. Displacement curves for yohimbine (●) and prazosin (○) vs 10 nM [^{3}H]dihydroergocryptine. Yohimbine displaced the bound radioligand from a single population of α-receptors whereas prazosin was ineffective. The similarity of the data for the two radioligands (also in Table I) indicates that [^{3}H]dihydroergocryptine binds to α_2-receptors in the islets.

pancreatic β cells inhibit insulin secretion whereas the β-adrenergic receptors stimulate it. With the availability of more specific adrenergic agonists and antagonists it has been suggested that the effects on insulin secreting responses are exerted by discrete subtypes of receptor population. Thus clonidine, which is an α_2-receptor agonist, has been shown to inhibit insulin secretion (7) and this response is blocked most effectively by the α_2-antagonists, e.g., yohimbine, and not by α_1-antagonists, e.g., prazosin (8).

The present study provides more direct measurements of adrenergic receptor density and affinity by using radioligands. Clearly, the data on both the displacement of [^{3}H]clonidine by the various agonists (Fig. 2) and by the two specific antagonists (Fig. 3) support the conclusion that the α-adrenergic receptors on the rat pancreatic islets are of the α_2 subtype.

The mechanisms whereby the various types of adrenergic receptors exert their effect on insulin secretion are unknown. Some suggestions have been made regarding their role in adipose tissues metabolism (9). In this tissue activation of β-adrenergic receptors stimulates adenylate cyclase activity and activation of α_2-receptors is believed to counter this stimulation. The general applicability of these mechanisms remains uncertain inasmuch as α_2-receptors have been reported only in human and hamster fat cells (10) but not in rat adipocytes (11).

Attempts to implicate cyclic AMP in the α-adrenergic inhibition of insulin secretion in rat pancreatic islets have given contradictory results. Although addition of clonidine to such cells clearly inhibited glucose-induced insulin secretion, there was no affect on adenylate cyclase activity (12). By contrast, it has also been reported that clonidine, epinephrine, and norepinephrine were effective in diminishing the glucose-induced accumulation of cyclic AMP in islet cells (8). The reason for the discrepant data is not clear.

It is of interest that glucose per se increases islet adenylate cyclase activity and results in accumulation of cyclic AMP in the islets. Likewise, stimulation of β-adrenergic receptors increases the adenylate cyclase and accumulation of cyclic AMP. Although the β-adrenergic receptor blocker, propranolol, decreases insulin secretion evoked by a glucose load (13) it is not known if there is a link between β-adrenergic receptors and insulin secretion evoked by glucose or whether the β-adrenergic system participates at some later stage in insulin secretion. Also it is conceivable that propranolol inhibited insulin secretion through its local anesthetic effect which is independent of its adrenergic blocking action.

Last, it has been reported, in abstract form, that addition of epinephrine to islets increased insulin secretion when glucose was absent from the media, but it decreased insulin secretion in the presence of glucose (14). We have found that presence or absence of glucose in the media has no effect on the α- or β-adrenergic receptor density or affinity (1, 2). Thus it appears likely that the factors which determine whether the adrenergic influence will increase or decrease insulin secretion are at sites beyond the adrenergic receptors.

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