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SPECIFIC RECEPTORS FOR SULFONYLUREAS IN BRAIN AND IN A B-CELL TUMOR OF THE RAT

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Attempts to demonstrate receptor binding of sulfonylureas, either in the pancreas [1,2] or in other organs [3], have failed so far, most likely because the methods chosen by the investigators were not suitable for the detection of a highly specific binding site. Although from a theoretical point of view the pancreatic B-cell has to be considered as the ideal organ for the demonstration of receptor binding of sulfonylureas, one has to take into account the difficulty of obtaining suitable amounts of islet material. Therefore, as a preliminary approach, a crude membrane preparation from rat brain was chosen as a model. Rat brain is being used quite extensively for receptor binding studies, of course predominantly for CNS-active compounds. However, it has recently been demonstrated that other compounds, yet to be shown to function in the brain, bind specifically to membranes of the brain [4,5]

MATERIALS AND METHODS

Preparation of cerebral cortex homogenates: Male rats (Chbb: THOM, SPF, 200 g) were decapitated, the cerebral cortex was prepared, immersed in ice-cold phosphate buffer (Soerensen, pH 7.5), and, under continued cooling with crushed ice, homogenized in an Ultra Turrax (Jahnke u. Kunkel) tissue homogenizer. The cell membranes were centrifuged at 50000 g, resuspended in fresh phosphate buffer and recentrifuged at the same speed. The resulting pellet was suspended in phosphate buffer, the final dilution being 1 : 50, based on the wet weight of the cortex. 1 ml aliquots of this cell suspension were used for the incubations.

Binding experiments: In the association/dissociation experiment the assay was performed in a 100 ml batch and 2 ml-aliquots were taken out at the time intervals shown in fig. 2. To study the influence of protein concentration on binding variable dilutions of the membrane homogenate were prepared. In all other experiments 1 ml-aliquots of the membrane suspension described above were incubated with 1 ml of phosphate buffer containing variable amounts of test substances and gliquidone tracer. The separation of membranes from supernatant was carried out by filtration through Whatman glass microfibre filters (G/FB, 2.5 cm). The filters were rinsed with 5 ml chilled phosphate buffer, the samples were diluted with 2 ml of chilled buffer, filtered and washed 3 times with 5 ml portions of the same buffer. The filters were immersed in Instagel (Packard) scintillation cocktail (12 ml), after 6 hours the vials were shaken briefly and the radioactivity was measured in a Packard Tricarb 3380 scintillation counter. Each point in the graphs represents the mean of 4 determinations.

Nonspecific binding is defined as the proportion of tracer bound to the membranes (number of counts) that cannot be displaced by a large excess (10 μM) of unlabeled gliquidone.

Specific binding is the proportion that is displaced by 10 μM gliquidone.

^{3}[H]-gliquidone was prepared by NEN, unlabeled gliquidone was synthetized by Dr. Griss (Dr. K. Thomae

GmbH), glibenclamide and tolbutamide were kindly provided by Dr. Heptner (Hoechst AG), and chlorpropamide by Dr. Kienzl (Boehringer Mannheim).

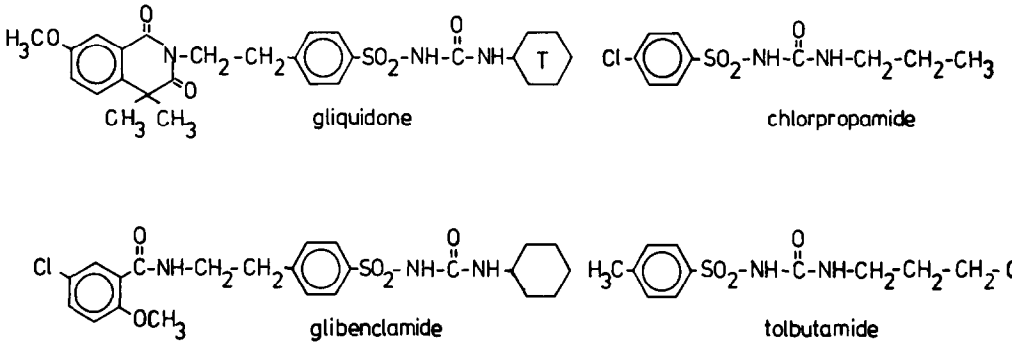

Fig. 1: Chemical structure of the sulfonylureas used in this study. The letter "T" in the formula of gliquidone indicates the position of the tritium label

RESULTS

Fig. 2 shows the time-course of the association and dissociation of labeled gliquidone (1-cyclo-hexyl-3-[p-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2-(1H)-isoquinolyl)-ethyl]phenyl]sulfonylurea) to binding sites in a crude preparation of membranes from rat cerebral cortex. Tritium labeled gliquidone was added to a suspension of membranes and the course of the association reaction was followed by determining the degree of binding in aliquots of this suspension. After 1 hour a large excess of unlabeled gliquidone was added and the degree of binding was followed for another 2 hours. The curves in fig. 2 represent the nonspecific binding and the specific binding. Under the conditions of the experiment the association reaction between 0.9 nM ^{3}[H]-gliquidone and it's binding site is characterized by a half life of about 15 minutes. The dissociation half life is between 60 and 70 minutes.

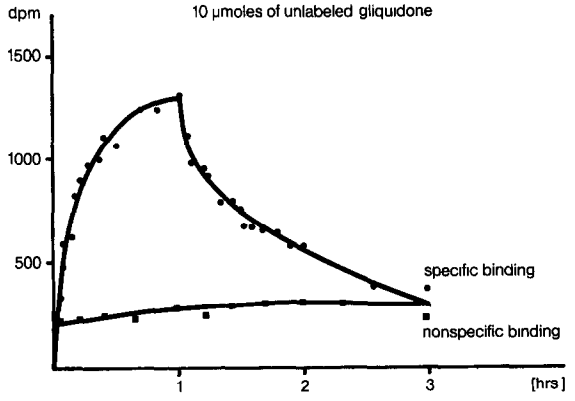

Fig. 2: Time course of association and dissociation of 0.9 nM ^{3}[H]-gliquidone to rat cortical membranes

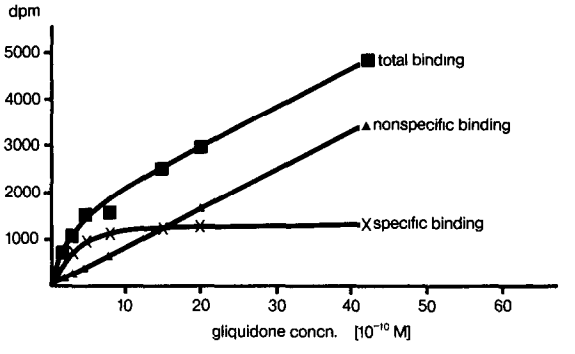

Fig. 3: Influence of tracer concentration on binding of ^{3}[H]-gliquidone to rat cortical membranes

The experiment depicted in fig. 3 was performed to assure that under the assay conditions chosen for the binding experiments, saturation phenomena due to high concentrations of gliquidone were negligible. Increasing concentrations of gliquidone were incubated at a constant protein concentration of 1 mg/ml. Whereas specific binding reaches a plateau at 1.5 nM gliquidone, nonspecific binding increases in a linear fashion up to 4 nM, the highest gliquidone concentration tested. In a separate experiment at a constant gliquidone concentration of 0.9 nM the protein concentration was increased stepwise in the incubation mixture up to a protein concentration of 4 mg/ml. It could be demonstrated that the protein concentration of 1 mg/ml used throughout this study was well within the linear portion of the curve and therefore suitable for the binding studies performed.

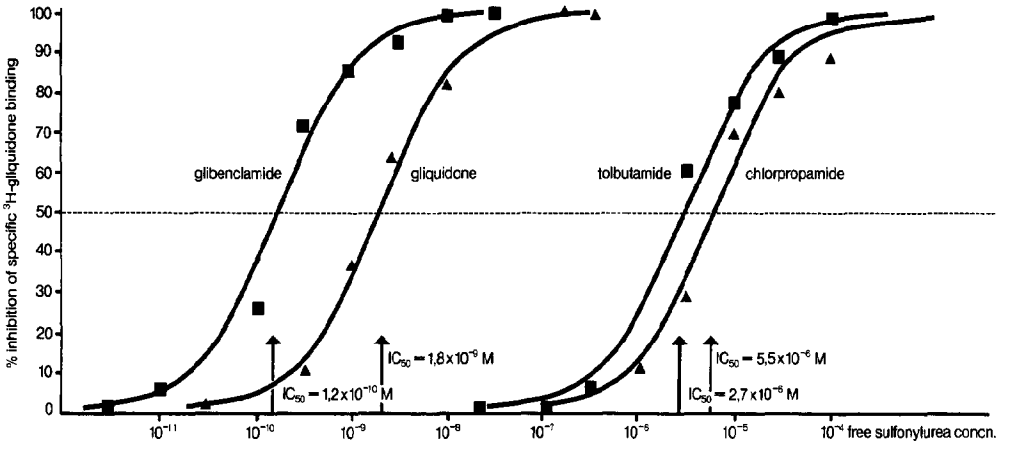

Fig. 4: Competition of glibenclamide, unlabeled gliquidone, tolbutamide, and chlorpropamide with the binding of 0.9 nM ^{3}[H]-gliquidone to rat cortical membranes

The next point of interest was, whether other sulfonylureas would compete for the same binding site as gliquidone. To answer this question the same concentration of ^{3}[H]-gliquidone as before, 0.9 nM, was incubated with increasing concentrations of glibenclamide, tolbutamide, and chlorpropamide in a suspension of membranes from rat cerebral cortex. As can be seen from fig. 4, the three other sulfonylureas compete with labeled gliquidone for its binding site. Using the IC_{50}-values estimated from the competition curves shown in fig. 4 the respective K_D-values were calculated. In tab. 1 these K_D-values are compared with the single doses recommended for therapy [6]. The table shows surprisingly clearly that compounds with a high K_D require higher doses to achieve metabolic control, in spite of the fact that such a comparison must be subject to considerable error due to differences between species and in pharmacokinetic properties.

	K_D [M]	therapeutic dose [6]
glibenclamide	$6 \cdot 10^{-11}$	2.5 - 15 mg
gliquidone	$9 \cdot 10^{-10}$	15 - 90 mg
tolbutamide	$1.4 \cdot 10^{-6}$	1 - 1,5 g
chlorpropamide	$2.8 \cdot 10^{-6}$	125 - 500 mg

Tab. 1: K_D-values for binding of some sulfonylureas to membranes from rat brain, using 0.9 nM ^{3}[H]-gliquidone as radioactive tracer, compared to therapeutic doses in man

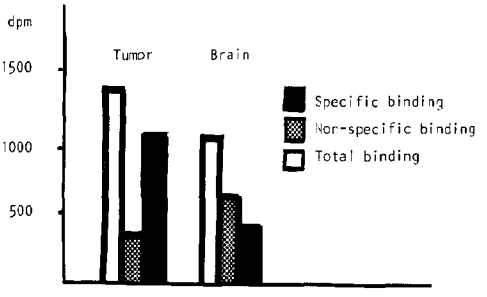

Fig. 5: Comparison between binding of gliquidone to rat cortical membranes and binding to membranes prepared from a ß-cell tumor of rat. Total, non-specific and specific binding are shown at a ^{3}[H]-gliquidone concentration of 0.83 nM in the presence of 5 nM unlabeled gliquidone

It was considered very important to make an attempt to demonstrate binding in a tissue more related to the ß-cell. The idea to use membranes obtained from isolated islets of Langerhans was abandoned for the moment because of the difficulty in preparing a sufficient amount of tissue. However, since a ß-cell tumor was available [7], an experiment was performed to determine whether labeled gliquidone binds to a membrane preparation from this tumor. Fig. 5 shows that, under comparable conditions, more ^{3}[H]-gliquidone is specifically bound to tumor membranes than to brain membranes. This may be a consequence of a higher receptor density in the tumor as compared to the brain. By contrast, no binding was observed to an analogous membrane preparation from a solid rat tumor of nonpancreatic origin (R.E. Offord, J. Gerlach, unpublished result).

DISCUSSION

In the previous section it was demonstrated that binding of ^{3}[H]-gliquidone has two components, a saturable one, that reaches a plateau at about 1.5 nM, and an unsaturable one, referred to as non-specific binding. The bound tracer can be displaced by cold gliquidone, providing a competition curve from which a K_D of about $9 \cdot 10^{-10}$ M can be calculated. The measurement of the kinetics of association and dissociation allows the estimation of kinetic constants which are of the same order to magnitude observed for other drugs. The value of K_D calculated independently, using these kinetic constants, is in excellent agreement with the value obtained above.

It is quite interesting to note that other insulinotropic agents compete for the same binding site as gliquidone. Glibenclamide which is used during antidiabetic therapy in doses approximately six times lower than gliquidone, has a K_D-value one order of magnitude lower than gliquidone. Both of the "first generation" sulfonylureas tested (which are applied in much higher doses during therapy) have K_D-values several orders of magnitude higher than gliquidone. These facts indicate a rough correlation between binding and biological activity, although it must be stressed that a pharmacological action of sulfonylureas in the brain has yet to be demonstrated.

All these observations make it quite likely that the results obtained reflect the binding of the compounds used in this study to a sulfonylurea receptor. The additional finding that gliquidone binds also to a ß-cell tumor, a tissue that resembles the ß-cell much more closely than the brain does, increase the probability even further that the observations described in this communication constitute the first evidence for a sulfonylurea receptor. Further work to characterize the receptor is in progress.

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