Determination of K_D-values of various fluorobenzylated Glibenclamide derivatives

R. Schirrmacher[a], A. Korobeinikov[a], A. Schneider[b], M. Schwanstecher[c], F. Rösch[a]
[a]Institut für Kernchemie, Johannes Gutenberg-Universität Mainz; [b]1. Med. Klinik, Johannes Gutenberg-Universität Mainz; [c]Institut für Pharmakologie und Toxikologie, Universität Braunschweig

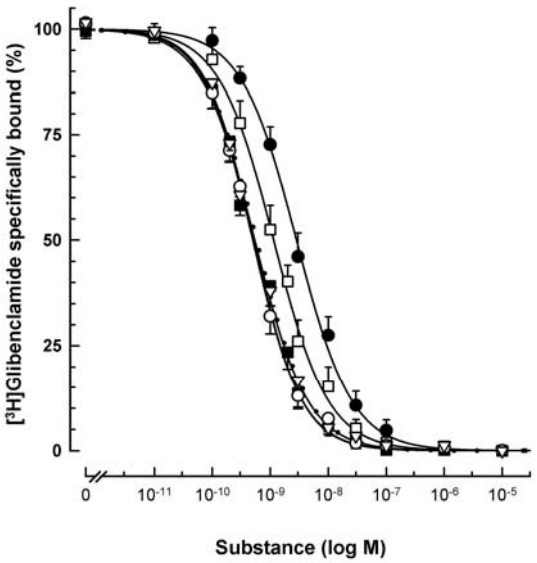
Aim. The present study was conducted to obtain reliable binding affinity data about some novel fluorobenzylated glibenclamide derivatives. These data are important for finding the most suitable compound among a great variety of new fluoroalkylated and fluorobenzylated glibenclamide derivatives for the use as PET ligands for the quantification and visualisation of the sulfonylurea receptor (SUR1). The new fluorobenzylated derivatives show a high binding affinity for SUR1 which is a prerequisite for further investigation.

Materials. [^{3}H]glibenclamide (specific activity 51 Ci mmol^{-1}) was purchased from NEN (Dreieich, Germany). Stock solutions of all drugs were prepared in KOH (50 mM) or dimethyl sulfoxide with a final solvent concentration in the media below 1 %.

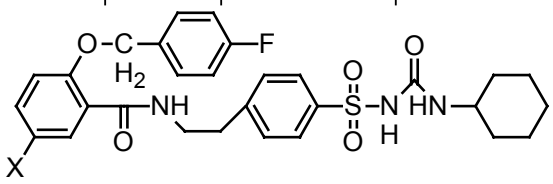
Binding assays. Transfections and membrane preparations were performed as described (Schwanstecher et al., 1992, 1998). Briefly, COS-1 cells cultured in DMEM HG (10 mM glucose), supplemented with 10 % fetal calf serum (FCS), were plated at a density of 5 x 10^5 cells per dish (94 mm) and allowed to attach overnight. 200 µg of pECE-human SUR1 complementary DNA (GenBank NP_000343) were used to transfect 10 plates. For transfection the cells were incubated 4 hours in a Tris-buffered salt solution containing DNA (5 - 10 µg/ml) plus DEAE-dextran (1 mg/ml), 2 min in HEPES-buffered salt solution plus dimethyl sulfoxide (10 %) and 4 hours in DMEM-HG plus chloroquine (100 µM). Cells were then returned to DMEM-HG plus 10 % FCS and were used 60-72 hours post transfection to prepare membranes as described (Schwanstecher et al., 1992). To measure binding to membranes from COS-cells the resuspended fraction (final protein concentration 5 - 50 µg/ml) was incubated in "Tris-buffer" (50 mM, pH 7.4) containing [^{3}H]glibenclamide (final concentration 0.3 nM, nonspecific binding defined by 1 µM glibenclamide) and other additions as shown in the figure. Incubations were carried out for 1 h at room temperature and were terminated by rapid filtration through Whatman GF/B filters.

Data. Half-maximally inhibitory drug-concentrations (IC_{50} values) and Hill coefficients (n) were estimated by fitting the function $B = 1/(1+([drug]/IC_{50})^n)$ to the data of each single diplacement experiment. K_Ds were calculated from IC_{50} values as described (Schwanstecher et al., 1992). Data shown as means ± S.E.M.

Results: Competition binding experiments were performed to assess the affinity of the glibenclamide analogues for binding to human SUR1 (figure). Unlabelled glibenclamide and substances 1-5 induced complete monophasic inhibition curves with Hill coefficients close to 1 (0.91 - 1.04) yielding dissociation constants (K_Ds) of 0.28 nM, 0.25 nM, 0.55 nM, 1.37 nM, 0.24 nM and 0.28 nM , respectively (figure; table).



X	entry	K_D (nmol)	nH
	Glyburide	0.28 ± 0.02	0.96 ± 0.03
Cl	S1	0.25 ± 0.03	1.04 ± 0.03
F	S2	0.55 ± 0.11	0.92 ± 0.04
H	S3	1.37 ± 0.25	0.91 ± 0.04
Br	S4	0.24 ± 0.01	1.02 ± 0.04
I	S5	0.28 ± 0.02	0.96 ± 0.03



The determination of K_D values of different substituted glibenclamide derivatives with less lipophilic properties is currently under investigation. The final aim of this study is to label the most suitable ligand with fluorine-18 in order to determine the beta-cell mass of the endocrine pancreatic tissue *in vivo* with positron emission tomography (PET).

References: Schwanstecher M, Brandt Ch, Behrends S, Schaupp U and Panten U (1992) *Br J Pharmacol* 106: 295-301. / Schwanstecher M, Sieverding C, Dörschner H, Gross I, Aguilar-Bryan L, Schwanstecher C, Bryan J (1998) *EMBO J* 17:5529-5535.