Synthesis and in vitro evaluation of \((S)-2-(\text{[}^{11}\text{C}\text{]}\text{methoxy})\)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl]-benzoic acid (\([^{11}\text{C}]\text{methoxy-repaglinide}\)): a potential \(\beta\)-cell imaging agent

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Abstract—The \(^{11}\text{C}\)-labeled sulfonylurea receptor 1 (SUR1) ligand \((S)-2-(\text{[}^{11}\text{C}\text{]}\text{methoxy})\)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl]-benzoic acid (\([^{11}\text{C}]\text{methoxy-repaglinide}\)) was synthesized in an overall radiochemical yield of 35\% after 55 min with a radiochemical purity higher than 99\%. This compound is considered for the noninvasive investigation of the SUR1 receptor status of pancreatic \(\beta\)-cells by positron emission tomography (PET) in the context of type 1 and type 2 diabetes. The specific activity was 40–70 GBq/\(\text{mol}\). In vitro testing of the nonradioactive methoxy-repaglinide was performed to characterize the affinity for binding to the human SUR1 isoform. Methoxy-repaglinide induced a complete monophasic inhibition curve with a Hill coefficient close to 1 (1.03) yielding a dissociation constant \((K_D)\) of 83 nM and an \(\text{IC}_{50}\) of 163 nM. Insulin secretion experiments on isolated rat islets were performed to prove biological activity, which was determined to be in the same range as that of original repaglinide.

Insulin secretion is regulated by the membrane potential of the \(\beta\)-cell, which depends on the activity of ATP-sensitive \(K^+\) channels (\(K_{\text{ATP}}\) channels) in the plasma membrane.\(^3\) Closure of \(K_{\text{ATP}}\) channels due to a rise of the cytoplasmic ATP/ADP ratio results in a depolarization of the membrane and in opening of voltage-sensitive \(\text{Ca}^{2+}\) channels. The increase in cytoplasmic \(\text{Ca}^{2+}\) stimulates the exocytosis of insulin.

\(K_{\text{ATP}}\) channels are composed of a small inwardly rectifying \(K^+\) channel subunit (Kir6.1 or Kir6.2) plus a sulfonylurea receptor (SUR1, SUR2A or SUR2B) belonging to the ATP-binding cassette superfamily.\(^4\) SURs represent the target for hypoglycemic sulfonylureas, a group of well-known antidiabetic agents, which have been in clinical use for years. Repaglinide is a novel fast acting prandial glucose regulator with a short plasma half-life (<1 h).\(^5\) Different from the sulfonylurea class, repaglinide is the first member of the carbamoylmethylbenzoic acid chemical family to be used in a clinical setting, being a new chemical class of insulin.

Keywords: \(\beta\)-Cell imaging; Repaglinide; SUR-receptor.

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secretagogues with an insulin release profile, which is very different to sulfonylureas like glibenclamide. The development of radioligands, which bind avidly to subtypes of K^+ channels is an important task for K^+ channel research. Recently, enantiomerically pure \[^{14}C\]repaglinide was used to obtain in vivo data about its metabolism and way of excretion. A detriment of this study was the low specific activity (2–50 Ci/mmol) of the radiotracer and additionally the use of carbon-14 whose radiation characteristics (short range \(\beta^\) particle, half-life \(t_{1/2} = 5730\) a) preclude a noninvasive investigation. Noninvasive investigations may become available by using a radioactive analogue with high specific activity and labeled with a positron emitting radionuclide, for example, \(^{11}C\) or \(^{18}F\). Positron emission tomography (PET) is a promising imaging technique to quantitatively assess biodistribution of radiolabeled pharmaceuticals noninvasively in humans and animals in vivo. This method is predestined to quantify and visualize the receptor status of diverse receptor systems. Radiolabeled sulfonylureas such as \(^{18}F\)-labeled glibenclamide and tolbutamide derivatives have been described and reviewed recently. These compounds have been proven to be unsuitable for \(\beta\)-cell quantification by Shiue and co-workers. Recently Sweet et al. performed a systematic screening of potential \(\beta\)-cell imaging agents such as glibenclamide, tolbutamide, serotonin, L-DOPA, dopamin, nicotinamide, fluorodeoxyglucose, and fluorodithizone and concluded that none of these compounds were likely candidates providing sufficient specificity for quantification with PET. Most recently our group investigated a promising \(^{18}F\)-labeled derivative of repaglinide whose accumulation in rat pancreas could be displaced by repaglinide, indicating specific binding of that radiolabeled compound. The pancreatic tissue displayed a stable and fast accumulation of radioactivity from 10 to 30 min p.i.

Unfortunately, the \(K_D\) value of the fluorine-labeled repaglinide derivative was 2.8-fold decreased versus repaglinide (142 ± 6 and 50 ± 4 nM, respectively; \(n = 5\) each; \(P < 0.05\)) indicating that \(^{18}F\)-derivatization leads to a decrease of the binding affinity (Fig. 1). In order to obtain a compound with higher affinity than the \(^{18}F\)-derivative we synthesized the \([^11C\]methoxy derivative 3 of repaglinide. Derivatization in terms of methylation is supposed to induce less alteration in affinity and pharmacology as compared to fluoroalkylation. With regard to the \(^{11}C\)-labeling, introduction of a \(^{11}C\)-methyl group is simpler to handle than a \(^{11}C\)-ethylolation, which would provide the structurally unchanged \(^{11}C\)-repaglinide. Fortunately, the analogous nonradioactive methoxy derivative 2 revealed an affinity significantly higher (83 ± 5 nM; \(n = 5\)) than the fluorinated derivative (\(P < 0.05\)) and similar to repaglinide itself (Fig. 1). The higher affinity (\(K_D\) 83 nM vs 142 nM) suggests the \(^{11}C\)-repaglinide derivative to be more suitable for receptor visualization than the \(^{18}F\)-derivative. The shorter physical half-life of \(^{11}C\) (\(t_{1/2} = 20\) min) in comparison to \(^{18}F\) (\(t_{1/2} = 109\) min) does not appear as a drawback since repaglinide rapidly accumulates within the pancreas reaching its maximum 10–30 min p.i.

Figure 1. Binding affinities of repaglinide and the novel repaglinide derivatives for human SUR1. A: Structures of original repaglinide, fluoroethylated repaglinide, and methoxy-repaglinide. B: \[^{1}H\]glibenclamide (0.3 nM) displacement assays were done with membranes from COS-1 cells transiently expressing human SUR1. All incubations were performed in Tris-buffer (50 mM, pH 7.4) containing displacing drugs as indicated. The IC\(_{50}\) values (half-maximally inhibitory concentrations) and Hill coefficients are: 106 ± 7 nM, 1.03 (repaglinide, □); 163 ± 9 nM, 1.03 (methoxy-repaglinide, □); 281 ± 11 nM, 1.01 (fluoroethylated repaglinide, ○). In parallel controls displacement by unlabelled glibenclamide was assessed (IC\(_{50}\) = 0.61 ± 0.03 nM, Hill coefficient = 1.00, data not shown). Results shown as mean ± SEM (\(n = 5\)). \(K_D\)s were calculated from IC\(_{50}\) values as described.

A \(^{11}C\)-labeled repaglinide derivative with high specific activity might thus become a valuable tracer probe for the visualization and quantification of human pancreatic \(\beta\)-cell mass in vivo.

### 1. Chemistry

The nonradioactive ‘standard’ compound (S)-2-(methoxy)-4-[3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl]-benzoic acid (methoxy-repaglinide) (2), required for in vitro evaluation and analytical purposes, was synthesized from the precursor (S)-2-hydroxy-4-(3-methyl-1-(2-piperidin-1-yl-phenyl)-butylcarbamoyl)-methyl-benzoic acid methyl ester (1) and methyl iodide in refluxing acetone with \(K_2CO_3\) as a base following a similar procedure as described previously (Fig. 2). Finally, the methylester moiety was cleaved with NaOH (1 N) in
refluxing methanol. Enantiomeric purity was proven by chiral high-performance liquid chromatography (HPLC) (Fig. 2).

For radioactive synthesis, the \(^{13}C\) isotope was produced via the \(^{14}N(p,\alpha)\(^{13}C\) nuclear reaction. The \(^{13}C\)methyl-iodide was synthesized with an automated synthesis module (PETtrace MeI MicroLab, GE Medical Systems) within 12 min. To 1 (2.1 mg, 4.8 \(\mu\)mol) dissolved in 250–350 \(\mu\)L DMF, 1N NaOH solution was added (4.8 \(\mu\)L, 4.8 \(\mu\)mol) and the mixture was heated at 100 °C for 1 min. A solution of \(^{13}C\)methyl-iodide (1.9–2.2 GBq) in DMF (350–450 \(\mu\)L) was added and stirred in a sealed reaction vessel at 100 °C for 2.5 min. The intermediate product was purified with HPLC (acetonitrile/0.1 M acetic acid/Na acetate buffer (pH=5) 8:2, flow rate 4 mL/min, \(t_r = 10.5\) min). After diluting the HPLC fraction containing the product with 15 mL water, it was loaded on a C18-SepPac cartridge (Waters) during 7 min, washed with 2 mL water, dried with nitrogen for 1 min and eluted with 1.5 mL ethanol to yield the intermediate (\(S\))-2-(\(^{13}C\)methoxy)-4-(3-methyl-1-(2-piperidine-1-yl-phenyl)-butyl-carbamoyl)-benzoic acid methyl ester. 1N NaOH solution was added (100 \(\mu\)L, 100 \(\mu\)mol) and stirred in a sealed reaction vessel at 100 °C for 10 min. The mixture was neutralized with 1M HCl (100 \(\mu\)L, 100 \(\mu\)mol). The product was purified by diluting with 15 mL water, loading on a C18-SepPac cartridge (Waters), drying with nitrogen for 1 min and eluting with 1 mL warm ethanol to yield 220–250 MBq. The product was purified by chiral high-performance liquid chromatography (HPLC) (Fig. 2).
and incubated for 10 min at 37 °C in the collagenase solution. Mechanical disruption of the digested pancreatic tissue was achieved by further incubation at 37 °C for 10 min in collagenase solution, interrupted every 2 min by shaking for 30 s. The digestion process was stopped by the addition of cooled HBSS plus 10% fetal calf serum (4 °C). Islet purification was achieved using a discontinuous three-phase Ficoll density gradient (densities: 1.090, 1.077, and 1.040). Islets were cultured in RPMI medium (Biochrom KG, Berlin, Germany) at 37 °C. The medium contained 5.1 mmol/L (1 g/L) D-glucose, 25 mmol/L HEPES, 10% fetal calf serum (Greiner Laboratories, Frickenhausen, Germany), 0.2 g/L Glutamax (GibcoBRL, Paisley, Scotland) and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin; GibcoBRL, Paisley, Scotland) and 10 μg/mL CiproBay; Bayer, Leverkusen, Germany). For each sample, 10 islets were picked (equal in size and shape) in a culture-insert with a membrane of 12 μm pore size (Millicell PCF, Millipore, France) and incubated in a 24-well culture-plate (Falcon Multiwell, Becton Dickinson, USA). First, basal insulin secretion was tested by incubating the islets with normoglycemic culture media (RPMI 1640 + D-glucose 1 g/L + 10% FCS) for 1 h at 37 °C. After the culture period, the media were collected and stored at −20 °C. The inserts with islets were transferred to normoglycemic culture-medium containing 0.10 μM of repaglinide or its methoxy derivative 2 and incubated for a stimulation period of 1 h. As a positive control, several inserts with islets were cultured in hyperglycemic culture-medium (RPMI 1640 D-glucose 15 mmol/L + 10% FCS) only. For negative control, normoglycemic culture-medium (RPMI 1640 D-glucose 5 mmol/L + 10% FCS) lacking repaglinide or its methoxy derivative 2 was used. The insulin content of each probe was quantified by a rat-insulin ELISA (Mercodia, Uppsala, Sweden). Insulin secretion was expressed as insulin release per islet/h. The stimulation index (Table 1) was calculated by dividing insulin output during stimulation (15 mmol/L D-glucose or 5 mmol/L D-glucose + test substance) by insulin secretion during basal incubation (5 mmol/L D-glucose). The relative stimulation index was calculated by dividing insulin output during stimulation with 5 mmol/L D-glucose + test substance by insulin secretion during incubation with 15 mmol/L D-glucose. The increase of insulin secretion after stimulation with compound 2 was determined to be 2.0 ± 0.4 ng/islet/h and was in the same range as that of repaglinide (1.9 ± 0.4 ng/islet/h) (Table 1). These results indicate that the described derivatization of repaglinide does not alter its insulin-stimulating properties.

### Table 1. Insulin stimulating capacity of repaglinide and its methoxy-derivative 2

<table>
<thead>
<tr>
<th></th>
<th>Insulin releasea</th>
<th>Insulin releasb</th>
<th>Insulin releasc</th>
<th>Stimulation index [%]</th>
<th>Relative stimulation index [%]</th>
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<tbody>
<tr>
<td>Positive control</td>
<td>2.3 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>7.5 ± 3.3</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>Repaglinide N = 8</td>
<td>2.1 ± 0.7</td>
<td>4.0 ± 1.4</td>
<td>—</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>(2) N = 8</td>
<td>2.0 ± 0.6</td>
<td>4.0 ± 1.5</td>
<td>—</td>
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</tr>
</tbody>
</table>

Data are given as mean ± SE.

a ng/islet/1 h (5 mM glucose).

b ng/islet/1 h (5 mM glucose + 0.1 μM repaglinide or (2)).

c ng/islet/1 h (15 mM glucose).

3. Conclusion

[11C]Methoxy-repaglinide (3), a derivative of the SUR specific ligand repaglinide was synthesized as an enantio-merically pure compound with an overall radiochemical yield of 35% after 55 min with a radiochemical purity >99% (n = 8). The radioactive synthesis is easy to perform and the simple labeling with [11C]methyl-iodide is an advantage in comparison to a possible 11C-ethylation for obtaining the structurally unchanged repaglinide. In comparison to 18F-labeled repaglinide, the synthesis is more comfortable due to the availability of [11C]methyl-iodide via a commercially available synthesis module. In vitro evaluation studies of the non-radioactive methoxy analog 2 showed that this compound binds with high affinity to the human SUR1 receptor of the pancreas and biological activity was totally retained as proven by insulin secretion experiments.

Thus, further evaluations with [11C]methoxy-repaglinide are planned to elucidate its use for scientific and clinical studies using quantitative PET, although the imaging of β-cell loss is problematic due to the fainting imaging signal with progressing disease.

Acknowledgements

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References and notes


19. Compound 2 was purified with column chromatography (Si-60): solvent ethylacetate/n-hexane; [Rf = 0.55]. 1H NMR (400 MHz, MeOH-d4): δ 7.5 (d, 1H, J = 7.8 Hz), 7.2 (d, 1H, J = 7.8 Hz), 7.1 (m, 2H), 7.0 (t, 1H), 6.9 (s, 1H), 6.8 (d, 1H), 5.5 (m, 1H), 3.7 (s, 3H), 3.5 (s, 2H), 3.0 (m, 2H), 2.6 (m, 2H), 1.8–1.1 (m, 9H), 0.9 (m, 6H); 13CNMR (MeOH-d4): δ 171.9, 171.0, 157.5, 151.9, 139.5, 139.1, 129.7, 127.1, 125.7, 124.0, 123.7, 120.6, 120.3, 111.7, 54.6, 46.0, 42.5, 33.1, 26.3, 24.9, 24.5, 23.8, 22.0, 21.1; FDMS: m/z (%) = 439.3 (100%).

20. HPLC-column: quality control: (a) Phenomenex Luna 5 μm C-18 (4.6 × 250), flow: 1 mL/min, solvent: acetonitrile/0.1M acetic acid/Na acetate buffer (pH=5) 8:2, t1 = 13.0 min, t2 = 3.9 min, t3 (intermediate) = 9.6 min; (b) preparative purification: Phenomenex Luna 5 μm C-18 (10 × 250), flow: 4 mL/min, solvent: acetonitrile/0.1M acetic acid/Na acetate buffer (pH=5) 8:2, t1 = 16.1 min, t2 = 4.6 min, t3 (intermediate) = 10.5 min.


22. [3H]glibenclamide (specific activity 51 Ci mmol⁻¹) was purchased from NEN (Dreieich, Germany). Stock solutions of all drugs were prepared in KOH (50mM) or dimethyl sulfoxide with a final solvent concentration in the media below 1%.

