6 Summary

Aim: We believe that Positron Emission Tomography (PET) will be the modality, which will allow for the first time non-invasive determination of beta-cell mass (BCM) of the human pancreas in vivo, because of the very high sensitivity of radionuclide imaging (picomole). So far, the intention failed at the lack of a beta cell-specific contrast agent. The requirements for such an agent are due to the unfavorable mass ratio of beta to non-beta cells are rather high (e.g. favourable beta-cell specificity, rapid elimination, no toxicity, accumulation proportional to BCM, detect human beta-cells). Methods: To identify a beta-cell-specific contrast agent, a phage-based single-chain antibody (SCA)-library was selected, a) in rats in vivo, or b) on freshly isolated rat islets in vitro, or c) on a beta-cell line in vitro, to isolate islet specific SCA. Subsequently the isolated SCA were characterized in vitro and in vivo. Results: We isolated 5 SCA (SCA 1, 2, 5-7), which were selective for human beta-cells in situ, internalized in rat beta-cells in vivo and were located in the endoplasmatic reticulum and the insulin granules, as shown by electron microscopy. These SCA did not bind in vivo to other endocrine cells of the islets, exocrine and extrapancreatic tissues (e.g. liver). Furthermore, we could isolate 2 SCA (SCA 3 and 4), that bind highly selective to pancreatic alpha-cells. The identification of relevant target proteins is on the way. We have shown that the in vitro selectivity of SCA 1 for beta-cells compared with binding to alpha-or exocrine cells exceeds 500:1. These numbers are far in excess of the estimated signal-to-background ratio required for the reliable and selective determination of BCM with PET in vivo and therefore the SCA 1 has significant advantages over previously evaluated agents. The SCA 1 also shows a rapid (t1/2 = 8 min) and high-volume (> 650,000 SCA / beta-cell) cellular uptake in beta-cells. We postulate a membrane protein or receptor-driven process. Such mechanism would be consistent with previous studies in this area in other cell types and would also be consistent with the competitive binding characteristics of different SCA at beta-cells. Another important characteristic of the presently described SCA in favour a diagnostic application in vivo is the avid elimination of the unbound particles from the circulation (t1/2 = 22.7 min). In addition, in a biodistributions-study it could be demonstrate that the tagging intensity (measured with a gamma counter ex vivo) coming from [125I]-labeled SCA1 after i.v. application in rats was strictly proportional to the BCM and invers correlated with glucose excursions during an IPGTT. These promising results could be confirmed non-invasively in diabetic rats.
using PET. Finally, the presented SCA have no effect on islet cell function and turnover. **Conclusions:** The newly developed SCA fulfill not only *in vitro* all requirements of a beta-cell specific contrast agent, but also allow for the first time reliable non-invasive estimation of the BCM in rats *in vivo* using PET. These promising results must be confirmed in larger animals, to be hopefully applied in humans in the future.