13C and 31P NMR spectroscopy in perfused mouse liver: A robust functional assay for the assessment of glucagon receptor antagonists

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Introduction

The transition from *in vitro* studies to *in vivo* demonstration of compound efficacy is often confounded by animal dosing issues and/or less than optimal pharmacokinetic parameters. The perfused liver technique[1] in combination with the introduction of a 13C-labelled metabolic substrate and 13C NMR spectroscopy offers a real time, ex-vivo probe of the major metabolic pathways (glucose metabolism, TCA cycle, fatty acid metabolism) in excised, yet still functioning livers. Here, this technique is applied to perfused livers from mice expressing the human glucagon receptor to develop a valuable assay for assessing the efficacy of novel small molecule glucagon receptor antagonists for the treatment of diabetes.

Methods

Experiments were performed on a Bruker 11.7T (500MHz proton resonant frequency) wide bore NMR spectrometer using a 20mm TXO probe tuned for 13C and 31P observation. Mice were anesthetized in the middle of the dark cycle and, following a portal vein cannulation, livers were excised and placed in a custom 20mm NMR tube as part of the apparatus shown in Figure 1. ATP levels, measured via 31P NMR spectroscopy, were used to monitor hepatic viability over the course of the studies. In this setting ATP levels were unchanged (<5%) for approximately three hours. The biosynthesis of glycogen in the perfused liver was monitored by infusing [2-13C]pyruvate and following the 13C enrichment at C-1 of the glucosyl units in glycogen by 13C NMR spectroscopy. After a sufficient time (~90 min) to allow for the accumulation of 13C-glycogen, glucagon (50 pM) was added to the perfusion buffer in the presence or absence of novel glucagon receptor antagonists. Glucagon alone resulted in the immediate cessation of glycogen synthesis followed by the breakdown of 13C-glycogen to 13C-glucose. The degree to which this glycogenolysis was attenuated by glucagon receptor antagonists gave a direct measure of their efficacy.

Results

Figure 2 displays the time course of the integral of the [1-13C]glycogen peak for vehicle (DMSO) treated livers, livers treated with vehicle plus glucagon, or livers treated with glucagon plus one of three novel thiophene-derived glucagon receptor antagonists[2]. One notable aspect of this technique is that the difference between vehicle treated livers and vehicle plus glucagon treated livers results in a very large window over which to observe the effects of glucagon receptor antagonists. In previous *in vitro* functional assays, compounds **1** and **2** had an IC50 for inhibition of cyclic AMP production of 129nM and 34nM, respectively, while compound **3** showed no effect. Consistent with this data, compound **1** shows a modest inhibition of glycogenolysis and compound **2** shows a near complete blockade of glycogenolysis, while the inactive compound **3** shows no effect. Hence, this technique gives information for establishing chemical structure activity relationships (SAR) as well as intrinsic compound efficacy in the target organ of interest independent of pharmacokinetic considerations.

Conclusion

The perfused liver NMR technique has been employed to develop a robust and sensitive assay yielding data which is consistent with, but more physiologically relevant than *in vitro* studies, yet avoids the complications of traditional *in vivo* studies. This technique has proven to be a valuable tool for the evaluation of glucagon receptor antagonists.

References

1. Cohen, SM. In Research in Perfused Liver: Clinical and Basic Applications. Ballet, F. and Thurman, RG. Eds. John Libbey: London, 1991, Chapter 4.
2. Duffy, JL et al. Discovery of Novel Thiophene-Derived Antagonists of the Human Glucagon Receptor, 228th ACS National Meeting.

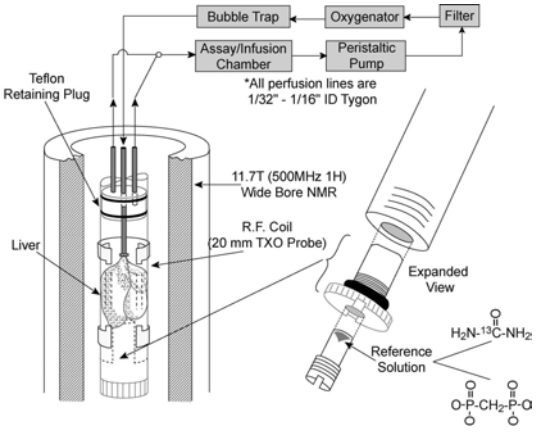

Figure 1. Essential components of the perfused liver apparatus. Inflow of perfusion buffer is via the portal vein cannula. The expanded view of the bottom of the NMR tube on the right shows an external reference solution which may be used for absolute quantitation.

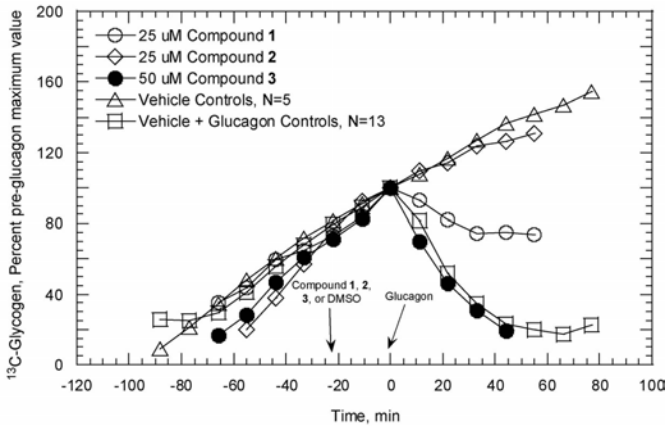

Figure 2. Time course of the integral of the [1-13C] glycogen peak for livers treated as described in Methods. SEM's are of similar size as the plot markers. The time of Glucagon addition is designated to be t=0.