Optimization of primary hepatocyte isolation for the pharmacological characterization of metabotropic glutamate receptor (mGluR) s ubtype 5: A study on Reduction

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Abstract

To minimize the number of animals used during experiments, it is important to choose the suitable enzyme according to the final goal. In our work, we demonstrated the superiority of collagenase IV in the maintenance of functional transmembrane receptor, thus the pharmacological activity, in isolated rat hepatocytes.

Introduction

In our previous studies in rat hepatocytes,[1,2] a significant variability in the responsiveness of mGlu5 receptor has been found among different hepatocyte preparations, forcing us to increase the number of rats to obtain statistically significant results. The most used enzyme in hepatocyte isolation is collagenase Type I from *Clostridium hystoliticum*. It should be noted that the majority of pharmacological studies on isolated hepatocytes are aimed to evaluate the function of intracellular cytochromes; on the contrary, mGluR5 is a transmembrane receptor. Our hypothesis was that collagenase I partially affected the function of mGluR5. The goal of this research was to compare mGluR5 expression and function in hepatocytes obtained from collagenases type I and type IV.

Materials and Methods

Hepatocytes from male Wistar rats (200-250 g) were isolated using collagenases I or IV (Worthington). After isolation, morphology, cell number and viability of rat hepatocytes were measured. mGluR5 protein expression was assessed by western blot analyses. mGluR5 activation was evaluated by inositol monophosphate (IP-1, Cisbio Kit) accumulation after treatment with the mGluR5 orthosteric agonist ACPD (Tocris) and the selective antagonist MPEP (Tocris).

Results

No difference in morphology, cell number and viability was observed when using collagenase I as compared with collagenase IV. A significant increase in mGluR5 protein expression was observed in hepatocytes isolated using collagenase IV with respect to collagenase I. Moreover, hepatocytes treated with ACPD and isolated using collagenase I presented lower levels of IP-1 when compared to the hepatocytes isolated by collagenase IV; in addition, hepatocytes isolated by collagenase IV and MPEP-treated showed a significant decrease in IP-1 with respect to hepatocytes isolated with collagenase I.

Discussion and Conclusions

According to our findings, rat hepatocytes isolated with collagenase IV are more suitable for pharmacological studies on mGlu5 receptor. These results suggest that collagenase IV better preserve the functionality of surface proteins. Collagenase I has been widely used for hepatocyte preparations for studies on hepatic cytochromes; cytochromes, being intracellular enzymes, are probably not affected by the collagenase used during the isolation process.

The use of collagenase IV may result in a higher consistency in the results, reducing the number of animals, especially in pharmacological studieson surface receptors.

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