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Effect of thiopental, pentobarbital and diethyl ether on early steps of insulin action in liver and muscle of the intact rat

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Abstract

A large number of experimental studies have investigated insulin signaling in rats. In these studies different anaesthetics have been used to anaesthetize rats. However, the direct effects of anaesthetics on the regulation of the early steps of insulin action are not known. In the present study, we investigated the effect of thiopental, pentobarbital and diethyl ether on the plasma glucose disappearance rate, IR, IRS-1 and IRS-2 tyrosine phosphorylation, IRSs association with PI 3-kinase, Akt and Erk phosphorylation, in liver and muscle of rats. Fasting plasma glucose levels were higher in animals anaesthetized with ether. No differences in plasma glucose disappearance rates were observed, however. Insulin-induced IR, IRS-1 and IRS-2 tyrosine phosphorylation, association of these substrates with PI 3-kinase and Akt and ERK phosphorylation were similar in the three groups of animals in both tissues. These data suggest that both thiopental and pentobarbital may be used in studies where changes in insulin signaling are being measured and where adequate general anaesthesia is required.

Keywords: Pentobarbital; Ether; Ethyl; Thiopental; Signal transduction; Phosphoproteins

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Introduction

Basal and insulin-stimulated glucose metabolism have now been extensively studied in intact rats. These studies are often performed in an anaesthetized preparation to eliminate the influence of environmental factors. In these studies, different anaesthetics have been used to anaesthetize rats and fragments of liver and muscle were excised and their insulin signaling measured (Choi et al., 2002; Hirata et al., 2003; Nunes et al., 2001; Patti et al., 1999; Qiao et al., 2002; Thirone et al., 2004). Barbiturates are the most widely used anaesthetic with established effects on lowering sympathetic nervous system (SNS) activity, thus altering hemodynamic parameters such as heart rate, stroke index, blood pressure, and total cardiac output (Baum et al., 1985; Lang et al., 1987; Wixson et al., 1987) and lowering core body temperature (Baum et al., 1985; Wixson et al., 1987). Despite the use of anaesthetized rats in many studies, there is still some uncertainty concerning the precise effects of anaesthesia on in vivo insulin action.

During the past decade, many of the proteins involved in insulin action have been defined at the molecular level. The insulin receptor is a protein tyrosine kinase which, when activated by insulin binding, undergoes rapid autophosphorylation and phosphorylates intracellular protein substrates, including insulin receptor substrates (IRSs–IRS-1 and IRS-2 are the most important) (Lavan et al., 1997; Sun et al., 1991, 1995) and Shc (Kovacina and Roth, 1993). Following tyrosine phosphorylation, the IRSs act as docking proteins for several Src homology 2 domain-containing proteins, including phosphatidylinositol 3-kinase (PI 3-kinase), Grb2, SHP2, Nck and Fyn (Folli et al., 1992; Kuhne et al., 1993; Saad et al., 1993; Skolnik et al., 1993; Yamauchi et al., 1995). Downstream to PI 3-kinase the serine threonine kinase, Akt, is activated playing a pivotal role in the regulation of various biological processes, including the regulation of the metabolic actions of insulin receptor activation (Brozinick and Birnbaum, 1998; Downward, 1998). In contrast, downstream to Grb2 there is activation of the mitogen-activated protein kinase (MAPK-ERK), which is important in the regulation of gene expression and cell growth (Jhun et al., 1995; Kim and Kahn, 1997; Sale et al., 1995).

The effects of different anaesthetics on the regulation of the early steps of insulin action are not known. In the present study, we investigated the effect of sodium thiopental, sodium pentobarbital and diethyl ether on IR, IRS-1 and IRS-2 tyrosine phosphorylation and association with PI 3-kinase, Akt and ERK phosphorylation in the liver and muscle of rats stimulated with insulin. We chose to compare these two anaesthetic with ether, since previous studies have shown that 1) the effects of ether anaesthesia on liver and skeletal muscle glycogen concentrations are similar to decapitation, cervical dislocation (Fregosi and Dempsey, 1986; Musch et al., 1989; Winder et al., 1983) and 2) the arterial blood gas and acid-bases status of the rat during ether anaesthesia most closely resembles that of the rat in the unanaesthetized state (Fregosi and Dempsey, 1986).

Materials and methods

Materials

Reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were from Bio-Rad (Richmond, CA, USA). Tris, phenylmethylsulfonyl fluoride (PMSF), aprotinin, dithiothreitol (DTT), Triton X-100, Tween 20, glycerol and bovine serum albumin