Effects of CO_2 pre-treatment at narcosis on energy charge levels in cell nuclei and whole tissue of rat liver

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INTRODUCTION

CO_2 can be used in rats and mice as an initial anaesthetic in order to facilitate handling and reduce stress before injections or other kinds of treatment of short duration. However, its suitability may be reduced by interference with physiological conditions like the energy status of the tissue. It is well known from studies concerning concentrations of nucleotides and related compounds in rat liver that several factors during tissue sampling are of great importance for the results. For example, the importance of rapid freezing of the specimens in order to avoid hypoxia and enzymatic degradation of the molecules has been emphasized (*Faupel et al.* 1972, *Eriksson* 1980). The effects of stress (*Ove et al.* 1967, *Faupel et al.* 1972) and of different anaesthetics on nucleotides and other compounds (*Faupel et al.* 1972, *Eriksson & Stråht* 1981, *Christensson & Eriksson* 1985) have also been reported.

The aim of the present study was to elucidate the effect of CO_2 treatment of rats on energy charge levels in their livers. Short term effects as well as a subsequent recovery during prolonged narcosis were investigated. As different ATP pools in nuclei and cytosol have been reported (*Rapaport* 1980) the possible role of such a compartmentalization was taken into account by separate analyses of cell nuclei isolated by a non-aqueous technique.

MATERIALS AND METHODS

Chemicals

Nucleotides for calibration were purchased from Sigma Chemical Co, USA. Briethal (methohexital) was from Lilly, Switzerland, and Mebumal (pentobarbital) from ACO, Sweden.

Animals and narcosis

Twelve male rats, Sprague Dawley (Anticimex, Sweden), weighing about 300 g and given food and water ad libitum, were used. Three different groups of four rats were studied. The first group was anaesthetized with Briethal, 8 mg/100 g BW intraperitoneally. With this short acting barbiturate the rats were completely anaesthetized after 4-5 minutes, at which time the livers were excised. Each rat of the second group was initially anaesthetized in an air-tight cage into which CO_2 was introduced until the animal was unconscious (about 20-35 seconds). The rats were then removed from the cage and given 5 mg/100 g BW of Briethal i.p. The livers were excised 4-5 minutes after the start of the CO_2 treatment. In the third group the animals were given CO_2 as described, but in order to obtain a prolonged narcosis, the subsequent anaesthetic was Mebumal (a longer acting barbiturate) given i.p. 5 mg/100 g BW. The livers were excised 15 minutes after the start of the CO_2 treatment. In a previous study (not published) we found no differences in the nucleotide profile between rats given Briethal and Mebumal narcosis respectively. Thus no group of rats receiving Mebumal only was used.

Tissue sampling.

The livers were rapidly freeze-clamped with a pair of tongs, precooled in liquid N_2. The frozen livers were broken in liquid N_2, lyophilized in a vacuum below 10^{-1} atm. at -50° C for 48 hours and stored at -80° C until further use.

Isolation of nuclei.

Isolation of nuclei was performed in anhydrous glycerol according to *Kirsch et al.* (1970) with some modifications (*Andersson et al.* 1987). The purity of the nuclear preparations was judged from the RNA/DNA ratios (*Siebert* 1967 and 1968, *Andersson et al.* 1987) and by light microscopy.

Chemical analyses.
Extraction of the acid soluble fraction in cold perchloric acid and the subsequent neutralization with KOH was performed according to Eriksson (1980). For the extraction from whole tissue, the frozen material was ground in a mortar and homogenized in cold perchloric acid in an ice bath for about 1 minute using a Potter Elvehjem homogenizer. Extraction from nuclear fractions required no such homogenization. The total extraction time was 20 minutes for both kinds of preparations. RNA was determined according to *Munro and Fleck* (1966) and DNA according to *Scott et al.* (1956) as modified by *Hinrichs et al.* (1964).
The separation of nucleotides was done by HPLC as described previously (Andersson et al. 1987). The separation was carried out in a Polyanion S I HR 5/5 column (Pharmacia Fine Chemicals, Sweden) with a 10-1000 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer at pH 6,5 using a linear gradient during 15 minutes and an isocratic flow during 2 minutes with the high concentration (Sjöberg & Kanje 1987).

Calculations.
The adenylate energy charge (AEC) was calculated using adenosine phosphate values from each separation directly in the following formula:

$$AEC = \frac{ATP + \frac{1}{2} ADP}{(ATP + ADP + AMP)}$$

The total amounts of adenosine phosphates were determined in nmoles/mg DNA.

RESULTS AND DISCUSSION

The total amounts of adenosine phosphates of whole tissue were similar in the three groups, about 2300 nmoles/mg DNA. In the nuclei, the values decreased in an unlinear fashion in relation to decreasing RNA/DNA ratios as described by *Andersson et al.* (1987). Energy charge values of cell nuclei and whole tissue are shown in Table 1. The energy charge in the first group (Briethal) was in agreement with previous findings for barbiturates (*Christensson & Eriksson* 1985, *Andersson et al.* 1987). After introductory CO_2 treatment followed by short

Table 1. Adenylate energy charge values of rat liver whole tissue and cell nuclei

Animal treatment			Adenylate energy charge	
Group No	Narcosis	n	Whole tissue	Cell nuclei
1	Briethal 4-5 minutes	4	0.782±0.041[a]	0.765±0.057[b]
2	CO_2 + Briethal 4-5 minutes	4	0.667±0.020[a]	0.671±0.034[b]
3	CO_2 + Mebumal 15 minutes	4	0.811±0.015	0.804±0.008

a: $p \leq 0.0005$
b: $p < 0.01$

Table values are mean values + S. D. in each group and are based on values of double samples from each rat.

Statistics: Student's t-test.

anaesthesia (4-5 minutes) before tissue sampling, a significant decrease in energy charge was found. When the CO_2 treatment was followed by a longer narcosis, however, the energy status returned to normal before excision of the livers. Consequently CO_2, used as an initial anaesthetic, produces only short-lived effects on the energy status of the liver. All nuclear preparations of the three different groups had energy charge values equal to their corresponding whole tissue preparations, although the RNA/DNA ratios were between 0.22 and 1.50, indicating nuclear fractions of various purity (*Siebert* 1967 and 1968, *Andersson et al.* 1987) Thus no compartmentalization between nuclei and cytoplasm was found regarding energy charge, which is in agreement with findings by *Ove et al.* (1967). However, the effects of a decreased energy status in the cell may be more or less pronounced in different compartments due to localization of various adenosine phosphate dependent functions (*Jones* 1984, *Aw & Jones* 1982 and 1985). Furthermore, different adenosine phosphate compartments which may be shown by experiments using radioactive precursors (*Rapaport* 1980), may not be revealed by a pure quantification. Nevertheless, our results indicate that the use of CO_2 as an initial anaesthetic in order to facilitate the handling of laboratory animals might be convenient in liver sampling except in cases where short term effects must be avoided.

Summary.
CO_2 can be used in rats and mice as an initial anaesthetic in order to facilitate handling and reduce stress before injections or other kinds of treatment of short duration. Interference with physiological conditions like the energy status of the tissue may reduce its suitability. The present study was undertaken in order to elucidate the effect of CO_2 treatment of rats on energy charge values in their livers.
Rats were anaesthetized with either Briethal (methohexital) alone or CO_2 + Briethal for 4-5 minutes or CO_2 + Mebumal (pentobarbital) for 15 minutes and energy charge values were determined in samples from whole liver and in liver celle nuclei, isolated by a non-aqueous method. CO_2 caused a significant decrease in energy charge which was detected after the short narcosis of 4-5 minutes. After 15 minutes the values had recovered. Energy charge was similar in whole tissue and cell nuclei.

Sammandrag.
CO_2 kan användas till råttor och möss som en inledande narkos för att underlätta hantering och minska stress före injektioner och andra slag av kortvariga behandlingar. Användbarheten kan dock minskas genom att CO_2 kan tänkas påverka fysiologiska förhållanden t.ex. vävnadens energitillstånd. Denna undersökning har gjorts för att belysa effekten av CO_2-behandling av råttor på energy charge värden i levern.
Råttor sövdes med antingen enbart Briethal (methohexithal) eller CO_2 + Briethal i 4-5 minuter eller CO_2 Mebumal (pentobarbital) i 15 minuter, och energy charge bestämdes i prov från hel lever och levercellkärnor, som isolerats med vattenfri teknik. CO_2 förorsakade en signifikant sänkning av energy charge 4-5 minuter efter behandlingen. Efter 15 minuter hade värdena återställts. Värdena var lika i hela celler och cellkärnor.

Yhteenveto/K. Pelkonen
Hiirille ja rotille voidaan käyttää esinukutusaineeksi hiilidioksidia helpottamaan käsittelyä ja vähentämäa6n stressiä ennen injektioita tai muita lyhytaikaisia käsittelyjä. Menetelmän vaikutukset fysiologisiin muuttujiin, kuten kudosten energiatilaan, saattavat vähentää sen käyttökelpoisuutta. Tämän tutkimuksen tarkoitus oli valottaa CO_2 – vaikutusta rotan maksan energiatilaan.
Rottia anestetisoitiin joko Brietalilla (methohexital) yksin tai CO_2 + Brietalilla tai CO_2 + Mebumalilla (pentobarbital) 15 minuutin ajaksi ja energiatasot mitattiin koko maksataa ja maksasolujen tumista, jotka oli eristetty vedettömällä menetelmällä. CO_2 aiheutti merkittävän energiatason laskun, joka oli havaittavissa lyhyen 4–5 minuuttia kestävän narkoosin jälkeen. 15 minuutin kuluttua arvot olivat palautuneet. Energiavaraus oli yhdenmukainen koko kudoksessa ja tumissa.

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