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Drug metabolism in suspensions of isolated rat liver cells at high pressure

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The effects of many narcotics, anaesthetics and tranquillizers are known to be profoundly reduced at elevated atmospheric pressure [1, 2]. The decreased response to drugs at high pressure has been explained as caused by altered function of excitable membranes [3–5]. However, high pressure is known to alter the activity of some enzyme systems [5]. The observed changes in drug effects could conceivably be at least partly due to an effect of high pressure on the pharmacokinetics of drugs. As an initial step to elucidate the pharmacokinetics at high pressure, we have studied the rate of oxidation and conjugation of some drugs (antipyrine, paracetamol, sulphadimidine, sulphanilamide and diazepam) in suspensions of isolated rat liver cells at 70 ATA (70 times the normal atmospheric pressure).

Primary suspensions containing parenchymal and nonparenchymal liver cells from male Wistar rats (200–280 g body weight, fasted for 12 hr), were prepared according to the procedure described by Berry and Friend [6] as modified in this laboratory [7, 8]. Aliquots of 15 ml cell suspension (in the diazepam experiments 35 ml) were transferred to Petri dishes containing one of the following drugs: [^{14}C]antipyrine (0.01 μCi/ml) and [^{3}H]paracetamol (0.1 μCi/ml) were added to obtain an initial concentration of 25 μg/ml in the incubates. The initial concentrations of sulphonamides and of diazepam were 30 μg/ml and 3.4 μg/ml, respectively. The zero time samples were drawn immediately before the start of compression; the final samples were drawn after 60 min. In the experiments with diazepam, the samples were drawn at zero time, and at 5, 10, 15, 20, 25 and 30 min.

The experiments were performed in a cylindrical steel 1.9 l. chamber. Heat was supplied by an external heating coil. The temperature was measured with a thermistor placed in the chamber atmosphere or in the cell suspension, which was placed in the air-filled chamber and compressed with helium to 70 ATA in 3 min. The chamber was continually shaken during the experiments. The decompression phase lasted 60 sec. Aliquots of suspensions from the same rats were used in pressure and control experiments, the latter were performed at 37° in air at normal atmospheric pressure.

During the compression phase, the temperature in the chamber atmosphere rose from 35° to 39° and stabilized after a few minutes at 37 ± 1°. In the suspension, the maximum temperature during the compression was 38.0°, and during the incubation the temperature was kept at 37 ± 0.5°. Samples of the suspensions under high pressure were obtained through a Hoke stainless steel miniature needle valve. The first 2 ml were discarded in order to drain the dead space of the tubing and the valve.

The viability of the cells was tested by measuring the exclusion of trypan blue and the incorporation of [^{14}C]valine into protein. [^{14}C]Valine 265 μCi/mole, uniformly labelled, was added at the start of the incubation to an initial concentration of 0.01 μCi/ml. Samples for the determination of [^{14}C]valine were drawn immediately before compression, and after 60 min incubation. The incorporation into proteins was measured as described by Mørland and Bessesen [8].

The concentrations of [^{14}C]antipyrine and [^{3}H]paracetamol in suspensions of liver cells were determined by the methods of Bakke *et al.* [9] and Cohen *et al.* [10] with modifications as described [11, 12]. Sulphadimidine and sulphanilamide were determined by the Bratton–Marshall method [13]. Diazepam was determined by gas chromatography [14].

The viability of the liver cells after 1 hr at 70 ATA, about 80 per cent, as judged by the exclusion of trypan blue, was not significantly different from that observed in the control experiments. The incorporation of [^{14}C]valine into cell protein in the pressure experiments was equal to the incorporation in the control experiments. It therefore appears that the liver cells in suspension survive under the conditions used in the present experiment without gross impairment of cellular metabolic functions.

The drugs chosen as test compounds in this study are metabolized by various oxidative and conjugative reactions in the rat. Recent experiments carried out in this laboratory have shown that similar metabolism of antipyrine, paracetamol, sulphadimidine and sulphanilamide takes place in suspensions of rat liver cells [11, 12, 15]. First order kinetics have previously been demonstrated for these four drugs at the cell concentrations and during the incubation periods employed [11, 12, 15], and in the present study the metabolism of diazepam also followed first order kinetics throughout the experiments at high pressure and in control experiments (data not shown). The effect of increased atmospheric pressure on the oxidation and conjugation of the test drugs in the liver cell suspensions is shown in Table 1. The rates of metabolism at 70 ATA were not significantly different from those observed at 1 ATA with any of the drugs tested.

Other investigators have studied the metabolism of various drugs at high pressures using subcellular fractions of liver cells. In some of these studies, the fractions were prepared from animals which had been exposed to a hyperbaric environment *in vivo*, and the subsequent incubation experiments were carried out at 1 ATA. Alternatively, the preparations were obtained from animals maintained at normal pressure, and the *in vitro* studies of drug metabolism were carried out at high pressure. By using the former approach increased *N*-demethylation of morphine was found in 9000 *g* rat liver supernatant from animals that had been exposed to 11 ATA for 4 hr [16] and increased *o*-dealkylation of *p*-nitroanisol was demonstrated in 9000 *g* liver supernatant from rats that had been exposed to 20 ATA for 12 weeks [17]. However, with

Table 1. The metabolism of drugs in suspensions of isolated rat liver cells at 1 ATA and 70 ATA

Drug	Number of experiments	Drug concn μg/ml	Cell concn* cells × 10^6/ml	Incubation time, min	Per cent metabolized ± S.D. 1 ATA	70 ATA
Antipyrine	7	25	7.1	60	27.0 ± 9.4	22.0 ± 6.0
Diazepam	9	3.4	3.7	30	89.0 ± 2.4	86.7 ± 3.3
Sulphanilamide	13	30	5.8	60	24.7 ± 6.0	27.0 ± 6.5
Sulphadimidine	13	30	5.8	60	6.8 ± 2.7	5.0 ± 2.5
Paracetamol	7	25	1.4	60	58.3 ± 23.4	56.9 ± 19.3

* Viable parenchymal cells at the start of the incubation.

the latter type of protocol, *N*-demethylation of morphine was unchanged in 9000 *g* liver supernatant from rats maintained at 1 ATA when incubated at 21 ATA [18].

In the present study we have failed to demonstrate any significant effect of high atmospheric pressure on the metabolism of drugs *in vitro*. The decreased effect of some drugs under high pressure cannot be explained by a general increase in drug metabolism at the cellular level. The impact of other pharmacokinetic factors needs to be clarified in order to answer the question of how pressure affects drug actions.

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Substrate activites for adenosine kinase and adenosine deaminase in relation to the cytotoxicities of some O^6-alkyl derivatives of 8-azainosine and 8-azaguanosine

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Ribonucleosides of O^6-alkyl-8-azahypoxanthines and 6-(alkylthio)-8-azapurines at low concentrations are toxic to cultured H. Ep. # 2 cells [1]. It was anticipated that these 8-aza derivatives would have properties similar to those of the corresponding purine nucleosides and, therefore, that they would be substrates for adenosine kinase (AK) (EC 2.7.1.20), and that lines lacking this enzyme would be resistant to their action [2]. However, two lines of H. Ep. # 2 cells lacking AK activity and highly resistant (200 to 1000-fold) to adenosine analogs, such as *S*-methyl-6-thiopurine ribonucleoside, showed relatively low degrees of resistance (5 to 20-fold) to O^6-methyl- and O^6-ethyl-8-azainosine [1]. Inhibition of the cells deficient in AK indicated either that the unphosphorylated nucleosides had activity or that there was a pathway other than direct phosphorylation for their conversion to cytotoxic nucleotides. Since O^6-methylinosine is a substrate for adenosine deaminase (ADA) (EC 3.5.4.4) [3, 4], it appeared that the action of ADA might be responsible for the activity of O^6-alkyl-8-azainosines in the kinase-deficient cells. The product of this reaction would be 8-azainosine, which is cytotoxic as a result of its conversion to 8-aza-IMP either by direct phosphorylation or by an alternative pathway not involving AK [5] (Fig. 1). Another possible metabolic fate (not shown in Fig. 1) for O^6-alkyl-8-azainosines is their conversion directly to O^6-alkyl-8-azahypoxanthines by the action of purine nucleoside phosphorylase. These bases were not available for study, but they would be expected to be non-toxic because the closely related thio compound, *S*-methyl-6-thio-8-azapurine, was not toxic to H. Ep. # 2 cells at a concentration of 300 μM [1].

To obtain evidence on the metabolism of these nucleosides, we have examined the activities of selected compounds as substrates for AK and ADA. We have reported earlier the qualitative observation that some of these compounds were substrates for these enzymes [6, 7], but it was desirable to obtain quantitative data, particularly to determine if the rate of removal of alkoxy groups by ADA was sufficient to have metabolic significance.

The study with AK was performed with preparations purified 135- or 263-fold from cultured H. Ep. # 2 cells. The reaction was measured by determining the amount of labeled monophosphate formed when the candidate nucleoside was incubated with the enzyme in phosphate buffer (pH 7.0)

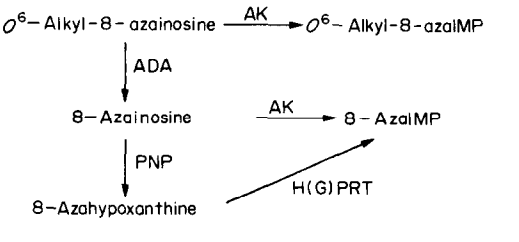

Fig. 1. Pathways for metabolism of O^6-alkyl derivatives of 8-azainosine. Abbreviations: AK, adenosine kinase; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; and H(G)PRT, hypoxanthine (guanine) phosphoribosyltransferase.