struction of cytochrome P-450. This protective effect of 3,4-benz(a)pyrene is actually due to the antioxidant properties of its hydroxylation products, and not to competition for sources of reducing equivalents in the NADPH-dependent electron-transport chain. This conclusion can be drawn from the following experiments. On incubation of hepatocytes in the absence of Fe^{++}–ADP, but in the presence of NADPH (10^{-4} M) very little accumulation of MDA was observed in the cells (0.20 ± 0.11 nmoles/10^6 cells), and cytochrome P-450 was correspondingly stabilized (Fig. 2). After addition of Fe^{++}–ADP ($5 \cdot 10^{-5}$ M) to the hepatocyte suspension, neither accumulation of MDA nor destruction of cytochrome P-450 took place during the next 30 min (Fig. 2). In suspension without benz(a)pyrene and without Fe^{++}–ADP, but with NADPH, MDA accumulation during the first 30 min was the same as in the system with addition of benz(a)pyrene, but in the 30 min after addition of Fe^{++}–ADP the MDA content increased by 1.5 times under these conditions.

Thus antioxidants of phenolic type, both exogenous and hydroxylation products formed as a result of oxidative metabolism of hydrophobic substrates, are effective protectors of cytochrome P-450 in liver cells against destruction due to lipid peroxidation.

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AFTEREFFECTS OF SHORT-TERM EXPOSURES TO HEAT OR COLD ON MONOAMINE OXIDASE ACTIVITY

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KEY WORDS: monoamine oxidase; hypothermia; hyperthermia; trace reactions.

Exposure for short periods to high or low temperatures is followed by the development of trace reactions [6]. There is evidence of lasting morphological and physiological changes in animals after exposure to hypo- and hyperthermia [1, 4]. Profound and lasting changes after exposure to brief but extreme cold have been described also at the level of the effector mechanisms of chemical temperature regulation [7]. However, it is not yet known whether aftereffects of exposure to high or low temperatures are preserved at the level of central mechanisms of temperature regulation. An important role in the regulation of body temperature is played by the serotoninergic system of the brain [8]. The principal enzyme participating in serotonin catabolism is monoamine oxidase (MAO), whose activity determines the intensity of serotonin destruction and can regulate its level in synaptic endings [2].

The aim of this investigation was to study the effects of exposure to different temperatures on the activity and catalytic properties of MAO.

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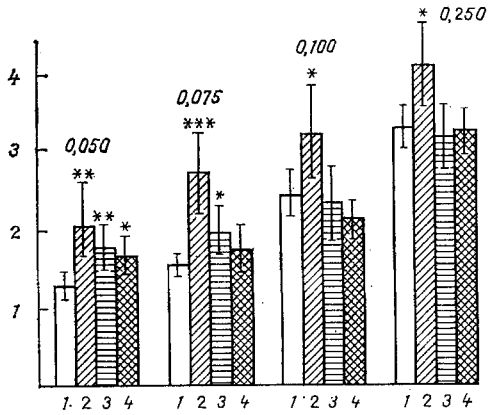

Fig. 1. Brain mitochondrial MAO activity (in nmole ammonia/2 mg protein/min) on deamination of serotonin in rats at different times after short-term hypothermia. Ordinate, MAO activity. Figures above columns denote serotonin concentration in incubated sample (in mM): 1) control, 2) 1 h, 3) 24 h, 4) 4 days after hypothermia. *P < 0.05 compared with control, **P < 0.01, ***P < 0.001.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-230 g. Animals of one group were exposed to short-term cooling in experimental constraining cages in a room with an air temperature of −15°C. Cooling of the animals for 1 h was accompanied by a fall of rectal temperature on average to 25°C. Rats of the other group were exposed to a high ambient temperature: 38°C for 1 h. Their rectal temperature was raised on average by 0.7°C. After the end of exposure to the different temperatures the rats were replaced in a room with an air temperature of 19-22°C and decapitated after various time intervals. A total mitochondrial fraction was obtained by differential centrifugation from a homogenate of the brain stem [11] and MAO activity in it was determined by a method based on measurement of ammonia set free during enzymic deamination of a substrate in an incubated sample [3]. Enzyme preparations were incubated with the substrate in 0.1 M Na,K-phosphate buffer, pH 7.4, at 37°C for 30 min. Activity of the enzyme was expressed in nmoles ammonia/mg protein/min. The protein concentration in the homogenate was determined by Lowry's method. Serotonin (serotonin creatinine sulfate, from Reanal, Hungary) was used as the substrate. The apparent Michaelis constant (K_m) was calculated from values of enzyme activity in the presence of different serotonin concentrations (six different concentrations from 0.05 to 1 mM were used) [5]. The maximal reaction velocity was determined in a saturating concentration of 3 mM serotonin.

EXPERIMENTAL RESULTS

The experiments showed that a single short exposure to cooling, accompanied by hypothermia, led to prolonged changes in the activity and catalytic properties of MAO. After the end of exposure to a low temperature MAO activity increased, and increased activity of the enzyme in the region of low concentrations of serotonin persisted for 4 days or more (Fig. 1). Changes also were observed in the kinetic parameter of the reaction: the value of K_m fell. The greatest decrease in K_m was observed 1 h after hypothermia: to 0.087 ± 0.013 mM (K_m in the control group of rats was 0.16 ± 0.019 mM, $P < 0.01$). The value of K_m was still low after 24 h (0.101 ± 0.023 mM, $P < 0.05$). After 4 days K_m was considerably restored but had not reached the control level (0.125 ± 0.015 mM, $P > 0.1$). The maximal reaction velocity was unchanged in all cases.

After short-term exposure to a high temperature, which was accompanied by moderate hyperthermia, a decrease in MAO activity was found. The greatest decrease in enzyme activity was observed 4 days after hyperthermia. This was followed by gradual recovery of MAO activity, but in the region of a serotonin concentration of 0.1 mM activity of the enzyme was still reduced after 7 days (Fig. 2). A small (not statistically significant) increase in K_m was found in the posthyperthermic period: K_m in the control was 0.153 ± 0.031 mM, 1 h after hyperthermia it was 0.218 ± 0.046 mM, and after 24 h it was 0.223 ± 0.038 mM ($P > 0.1$). The maximal reaction

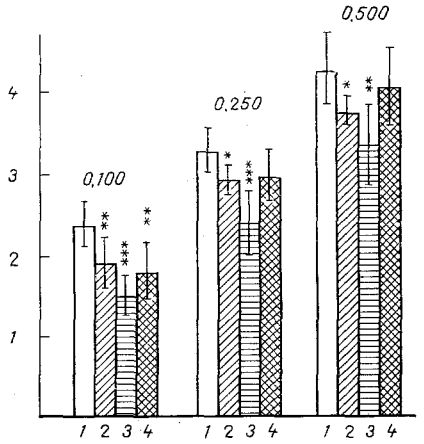

Fig. 2. Brain mitochondrial MAO activity (in nmole ammonia/mg protein/min) on deamination of serotonin in rats at different times after short-term hyperthermia: 1) control, 2) 1 h, 3) 24 h, 4) 7 days after hyperthermia. Remainder of legend as to Fig. 1.

velocity was changed in only one group of animals, namely 24 h after hyperthermia: from 5.6 ± 0.15 nmole ammonia/mg protein/min in the control to 6.4 ± 0.26 nmole ammonia/mg protein/min in the experiment ($P < 0.05$). After short-term hyperthermia changes were thus found in MAO activity in the opposite direction to those observed after short-term hypothermia, and they were distinguished by an even longer aftereffect.

Consequently, after a short-term exposure to both hypothermia and hyperthermia, even though the body temperature was restored and remained at a steady level thereafter, changes were observed in the activity and catalytic properties of MAO which persisted for several days. However, unlike short-term hypothermia, after the end of which the processes of serotonin catabolism intensified, to reach a peak after 1 h, and subsequently they remained high for 1-4 days, after the end of short-term hyperthermia MAO activity continued to fall, to reach a minimum after 4 days, and only after that was gradual recovery of MAO activity observed. In the region of the 0.1 mM concentration of serotonin, reduced activity of the enzyme was still preserved after 7 days. By contrast with the posthypothermic period, in which affinity of MAO for serotonin was increased, in the post-hyperthermic period some decrease in enzyme–substrate affinity evidently was observed, leading to a decrease in serotonin deamination in the region of low, near-physiological, serotonin concentrations. Hence a short exposure to both hypothermia and hyperthermia induces long-lasting changes, but in different directions and with different durations, in the system of serotonin catabolism in the brain. In the post-hypothermic period changes in MAO activity are directed toward increased destruction of serotonin, and they are evidently associated with the need to abolish the effect of serotonin as a factor inhibiting heat production in the body [10]. A rapid and considerable increase in enzyme activity is produced by an increase in the affinity of MAO for serotonin. The response to hyperthermia is characterized by opposite changes. Under the influence of high ambient temperatures activation of the serotoninergic system evidently takes place, accompanied by a decrease in brain MAO activity, which leads to potentiation of the effect of serotonin on heat elimination processes [11]. Changes in the activity and catalytic properties of MAO, which deaminates serotonin, discovered after a short exposure to both low and high ambient temperatures are probably a component of the trace reactions which arise in the temperature regulating system and they evidently lead to increased resistance of the body to subsequent exposures of the same type to high or low temperatures.

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REGULATION OF THE EXCISION REPAIR SYSTEM IN HUMAN CELLS CULTURED IN VITRO BY MEASLES VIRUSES, DEPENDING ON THEIR ATTENUATION

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Viruses may have a modifying effect on the excision repair system, either suppressing it [1, 2] or stimulating individual stages of this process. The effect of attenuated measles virus on the repair process is of practical as well as theoretical interest, because vaccination with attenuated virus is carried out annually on tens of thousands of children.

Accordingly, this paper describes the results of a comparative study of the effect of "wild-type" and attenuated strains of measles virus on different stages of excision repair induced by UV irradiation. Determination of activity of the system repairing DNA injuries produced by this mutagen is important because UV radiation is an ecologic factor to whose action vaccinated children are constantly exposed. Moreover, most chemical mutagens have an action of UV type, i.e., DNA repair requires involvement of basically the same enzyme systems. Consequently, information obtained with the use of UV radiation may be correct to some degree also for chemical substances of UV type which are widespread in the external environment.

EXPERIMENTAL METHOD

Experiments were carried out on a transplantable culture of human cells of line L-41. Two strains of measles virus were used: vaccine strain L-16 and the "wild-type" Edmonston strain. Cells were infected in suspension and the multiplicity of infection was 0.01-0.1 TCD_{50} per cell. As the source of UV radiation, two BUF-15 lamps (254 nm) were used. Activity of the initial stage of excision repair was investigated by a radiochromatographic method [5], whereby the number of thymine dimers formed during UV-radiation and their "excision" could be determined under postradiation conditions of incubation. With this aim, cells growing in a monolayer were irradiated 24 h after infection in a dose of 20 J/m^2. The content of thymine dimers in the cell DNA was determined immediately after irradiation and after 12 and 24 h of postradiation incubation. The number of thymine dimers was calculated by the equation:

$$\text{Number of dimers} = \frac{\text{Count in region of thymine dimers (in cpm)}}{\text{Count in region of thymine (in cpm)}} \times 100\%.$$

Reparative DNA synthesis activated by UV radiation was studied by a liquid scintillation method based on incorporation of [^{3}H]thymidine into the total mass of cells, with suppression of replicative DNA synthesis by hydroxyurea. The intensity of reparative synthesis was judged from the value of the stimulation index (SI), which is the ratio between the radioactivity counts (in cpm) in the irradiated cells to the radioactivity count in unirradiated cells.

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