the time taken for eosinophilia to develop to 15 h. Calculation of the efficacy of action of isadrine and Inderal in adrenalin-induced heart injury gave values A = 0.62 for isadrine and A = 2 for Inderal, i.e., the former potentiates the harmful action of adrenalin whereas the latter has a protective action.

The investigations thus showed a regular relationship between the efficacy of action of the two drugs tested in adrenalin-induced heart injury and changes in the duration of phases of responses of the PAS, as reflected in an increase or decrease in the time required for eosinophilia to develop in response to injection of adrenalin.

In the writers' view, this principle of evaluation of the efficacy of drug action can be used to develop objective criteria characterizing the action of chemical compounds and pharmacological agents under a wide range of extremal conditions.

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EFFECT OF SODIUM HYDROXYBUTYRATE ON THE AMMONIA CONTENT IN RAT MUSCLES DURING PHYSICAL EXERTION

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In experiments on rats forced to swim while carrying a load, sodium hydroxybutyrate was found to have a normalizing effect on the ammonia content in the striated muscles, a biochemical indicator of physical fatigue. Whereas in control rats not receiving hydroxybutyrate swimming led to a marked (more than twofold) increase in the ammonia content in muscle tissue, in animals receiving prophylactic sodium hydroxybutyrate (one only or as a 2-week course) ammonia did not accumulate. It is suggested that by preventing the toxic effect of one of the end products of nitrogen metabolism, sodium hydroxybutyrate may alleviate the after-effects of muscular fatigue.

KEY WORDS: sodium hydroxybutyrate; physical exertion; nitrogen metabolism; striated muscle.

Sodium hydroxybutyrate has the property of increasing the resistance of the body to hypoxia [5, 15]. Analysis of the mechanism of this effect has shown that the compound reduces the degree of disturbance of oxidative processes induced by hypoxia [1, 6, 10] and also reduces the changes in nitrogen metabolism in nerve tissue characteristic of that state [2]. It has also been shown that if sodium hydroxybutyrate is given during repeated physical exertion separated by short intervals of rest a tendency is observed toward stabilization of working capacity at a certain level [4]. Hypoxia due to a deficient oxygen supply to the tissues, and the so-called motor hypoxia due to physical exertion, are known to have some common pathogenetic factors, one of

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TABLE 1. Effect of Sodium Hydroxybutyrate on Ammonia Concentration (in μmoles/g wet weight of tissue) in Soleus Muscle of Rats Undergoing Physical Exertion

Parameter	Intact animals (I)	Swimming (II)	Sodium hydroxybutyrate (single dose) + swimming (III)	Sodium hydroxybutyrate (repeatedly) + swimming (IV)	Sodium hydroxybutyrate (single dose) (V)
$M \pm m$	2,44±0,57	4,31±0,54	1,92±0,25	1,61±0,27	1,72±0,35
E_I %	—	+77%	−21%	−34%	−29%
	—	$P_{I-II}<0,001$	$P_{I-III}>0,05$	$P_{I-IV}<0,02$	$P_{I-V}>0,05$
E_{II}, %	—	—	−56%	−63%	—
	—	—	$P_{II-III}<0,001$	$P_{II-IV}<0,001$	—

Legend. E_I and E_{II} – changes (in %) relative to ammonia concentration in intact animals and in animals compelled to swim, respectively.

which is a disturbance of nitrogen metabolism. This disturbance is manifested, in particular, by the accumulation of ammonia in the tissues and blood, which is found in hypoxic hypoxia [2] and during excessive physical exertion [8] and is due to a combination of increased production and delayed fixation of ammonia [9, 11, 13].

With these facts in mind the present investigation was undertaken in order to study the effect of sodium hydroxybutyrate on the ammonia content in muscle tissue, a biochemical indicator of fatigue during physical exertion.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 g. The model of physical exertion used was to compel unadapted rats to swim in a bath of water at 26°C, carrying an additional load equivalent to 6% of the total body weight, until the onset of total fatigue. The animals were then decapitated and at the same time the left hind limb was amputated. The limb was frozen whole in liquid nitrogen, after which the soleus muscle was removed and its tissue homogenized in 5 volumes of 10% TCA solution and centrifuged at 3000g. The ammonia content in the supernatant was determined by the isothermic distillation method [12]. The rats studied were divided into the following series: intact animals not receiving the compound and not compelled to swim (I); animals swimming until maximal fatigue without receiving the compound (II); animals receiving a single dose of hydroxybutyrate of 500-1000 mg/kg 5 h before swimming (III), rats receiving the compound daily for 2 weeks in the same doses (IV), and animals receiving a single dose of hydroxybutyrate and not compelled to swim (V). The number of animals in each series was 12-15.

EXPERIMENTAL RESULTS

As the data in Table 1 show, the ammonia content in the tissue of the rat soleus muscle under normal conditions averaged 2.44 μmoles/g wet weight of tissue. Muscular fatigue, which under the experimental conditions used developed 15-18 min after the beginning of swimming, was accompanied by a definite increase in the free ammonia concentration in muscle tissue. Sodium hydroxybutyrate, if given to animals not undergoing mucular exertion, caused a small decrease ($P > 0.05$) in the ammonia content in muscle tissue. However, after prophylactic administration to animals undergoing physical exertion 5 h later, sodium hydroxybutyrate sharply reduced the free ammonia concentration in the muscles. This effect was clear after a single dose, but clearer still after repeated administration of the compound for 2 weeks.

During physical exertion of considerable magnitude, giving rise to fatigue, the rate of protein breakdown is known to be much higher than the rate of protein synthesis. This leads to a reduced content of certain proteins in the muscles, the transfer of their low-molecular-weight fractions, polypeptides, and amino acids into the blood stream, and an increase in the nonprotein nitrogen concentration in the muscles and blood. Intensification of protein catabolism is associated with substantial disturbances of the ATP balance in a muscle in a state of contraction for a long time. Another possible source of ammonia formation in the working muscle is the purine nucleotide cycle [3, 14]. An important cause of accumulation of free ammonia in hypoxia is a decrease in the rate of the ATP-dependent reaction of conversion of glutamate into glutamine. The ability of sodium hydroxybutyrate to reduce the ammonia concentration in muscle tissue a little, revealed by the experiments described above, is evidently connected with the fact that in the presence of an excess of γ-hydroxybutyric acid the formation of α-ketoglutarate – the substrate binding ammonia in the ATP-independent reaction of glutamate formation – is increased. The effect of sodium hydroxybutyrate is particularly conspicuous during sudden physical exertion causing accumulation of ammonia. There is evidence of the ability of sodium hydroxybutyrate to increase the respiratory and phosphorylating activity of the mitochondria [10] and to create an additional reserve of hydrogen acceptors, which are deficient in hypoxia [2].

By preventing the acidotic shifts characteristic of hypoxia and leading to disturbance of the activity of certain enzyme systems, including glutamine synthetase, sodium hydroxybutyrate may speed up the binding of ammonia accumulating under hypoxic conditions. The experimental data given above suggest that sodium hydroxybutyrate may prevent the development of the accumulation of toxic end products of nitrogen metabolism, one of the undesirable manifestations of excessive physical work.

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INTRAHEPATIC CIRCULATION OF ^{14}C-PHENAZEPAM AND ITS METABOLITES IN ALBINO RATS

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Excretion of phenazepam and its metabolites in the bile and its intrahepatic circulation were studied in two groups of rats: bile donors receiving an intravenous injection of ^{14}C-phenazepam (14 mg/kg) previously, and recipients into whose duodenum the donors' bile was introduced. Phenazepam (compound I), its free-hydroxy metabolites (compound II), and a metabolite with a hydroxyl group in the aromatic ring (compound III) were shown to be excreted in the bile of these animals. Hydroxyl derivatives are excreted in the bile in the form of glucuronides also. Compounds I and III and the glucuronide of compound III undergo intrahepatic circulation.

KEY WORDS: phenazepam; metabolism; intrahepatic circulation.

With the introduction of phenazepam, a tranquilizer of the 1,4-benzdiazepine series, into medical practice the study of its metabolic pathways in experimental animals and in man and the rate of its excretion is interesting.

The investigation described below was carried out to study the ratio of phenazepam metabolites, both free and conjugated with glucuronic acid, entering the bile of albino rats following direct administration of ^{14}C-phenazepam. The composition of metabolites involved in intrahepatic circulation in the experimental animals also was studied.

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