pattern of neuronal activity with two predominant lengths of ISI (1-30 and 100-200 msec) is the integration of interactions between NE and both α_1- and β-adrenoceptors.

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The Invertor Mechanism of Changes in the Plasma Membranes of Hepatocytes during Induction of Microsomal Monooxygenases in Adult and Old Rats

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Age-specific changes of some parameters of the state of the plasma membrane are studied for genetic induction of enzymes of microsomal oxidation. Changes of the state of plasma membrane of hepatocytes are shown to be associated with the synthesis of a specific intracellular regulator (invertor).

Key Words: *plasma membranes; microsomal oxidation; phenobarbital; aging*

In our previous studies hyperpolarization of the plasma membrane was shown to develop during activation of protein biosynthesis caused by different factors (action of a number of hormones, regeneration, blood loss) [7]. It has been established that, during exposure to insulin and testosterone, the development of hyperpolarization results from activation of Na,K-ATPase and is governed by the synthesis of specific intracellular genome-controlled regulators, which have been defined by us as invertors [8,10,11]. In the course of aging the pattern of the plasma membrane response to activation of protein biosynthesis alters [11]. A genetic induction of microsomal oxidation enzymes in the liver causes pronounced shifts in protein biosynthesis [1,4]. There are numerous reports that the pattern of genetic induction of microsomal monooxygenases markedly changes in the course of aging [5,12]. The aim of the present study was to investigate age-related specificities of changes of some parameters of the state of the plasma membrane for genetic induction of enzymes of microsomal oxidation and to elucidate the possible mechanism of these shifts.

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MATERIALS AND METHODS

The experiments were carried out on certificated male Wistar rats (6-8 months). Phenobarbital (PB)

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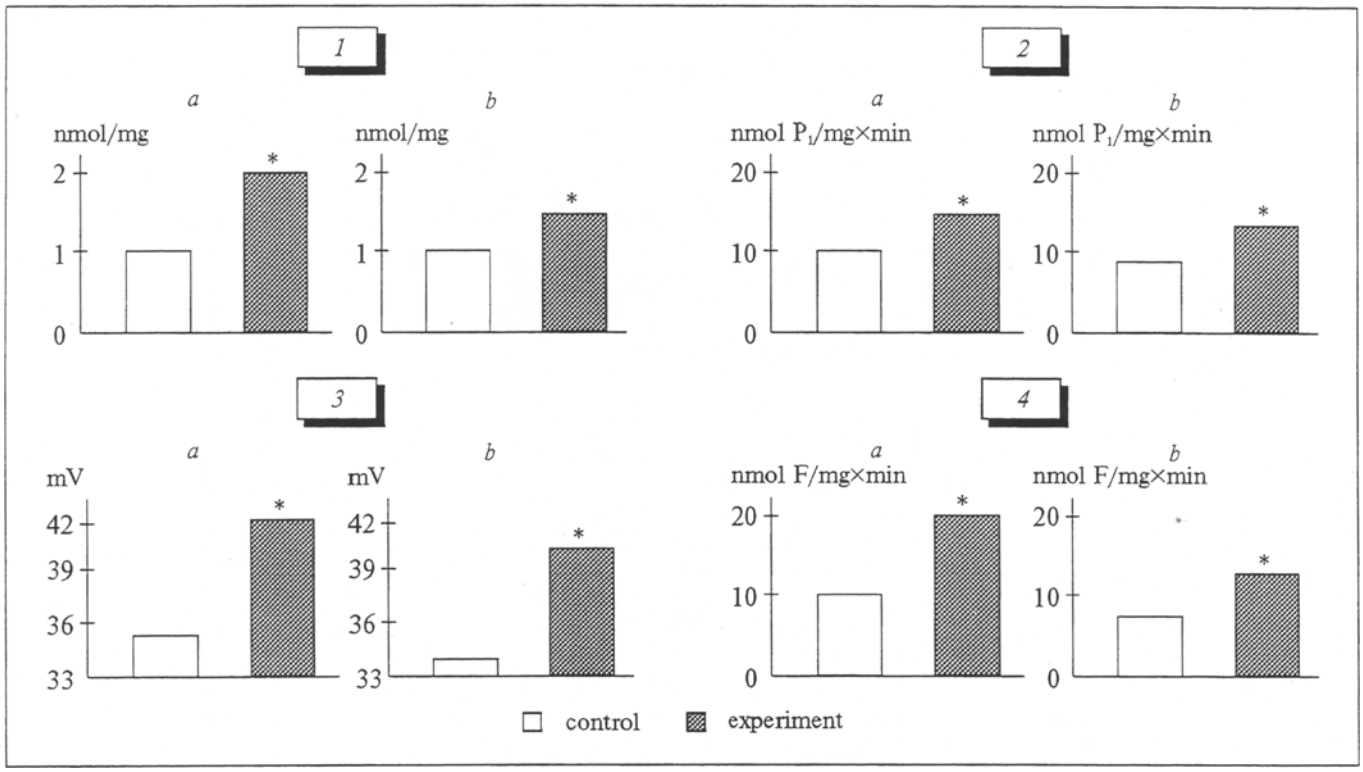

Fig. 1. Effect of PB on concentration of cytochrome P−450 (*1*), activity of aminopyrine demethylase (*2*), MP (*3*), and activity of Na,K−ATPase (*4*) of hepatocytes of adult (*a*) and old (*b*) rats. F: formaldehyde; P_i: inorganic phosphate. An asterisk indicates $p<0.05$.

was administered in a dose of 80 mg/kg intraperitoneally for 3 days. The content of cytochrome P-450 [15] and the aminopyrine demethylase activity [2] were determined in the microsomal fraction (105,000 g) of the liver. The fraction of isolated plasma membranes was obtained by a modified method [17]; its purity was assessed according to its enrichment (in comparison with homogenate) with 5'-nucleotidase, a marker enzyme of the plasma membranes [14]. Such an enrichment constituted, under the conditions of purification employed by us, approximately 15-20 times. The activity of Na,K-ATPase was assessed a the difference between the total activity and the Mg-ATPase activity [6]. The concentration of inorganic phosphates was determined as described elsewhere [16]. The fraction of isolated plasma membranes was incubated *in vitro* with the cytosol of hepatocytes and rat blood serum as follows: in the absence of ATP 150 μl of hepatocytic cytosol or serum and 100 μg of membrane protein were added to the reaction mixture for determination of the total or Mg-ATPase activity, and the tubes were placed on an ice bath with stirring for 40 min. ATP was then added to the tubes, and the ATPase activity was measured. The protein concentration in the samples was determined after Lowry [13]. The membrane potential (MP) was measured using microelectrode techniques [3].

RESULTS

In the course of aging, marked changes occur in the pattern of genetic induction of microsomal monooxygenases. As is shown in Fig. 1, after a three-day administration of PB, the P-450 content increases by 90% in adult animals and by 60% in old ones; the activity of aminopyrine demethylase increases by 68 and 57%, respectively. These shifts in genetic induction are realized against the background of changes of MP in the plasma membranes of hepatocytes. After a three-day administration of PB, the MP reliably increases (by 6.5 mV) in adult animals; in old rats this shift constitutes 4.5 mV. Presumably, this change of the MP is due to shifts in the active ion transport. In fact, under the conditions of genetic induction of microsomal monooxygenases the activity of Na,K-ATPase increases, its increment being different in animals of different age (Fig. 1). Thus, during the genetic induction of microsomal monooxygenases in both adult and old animals, shifts (an increase of MP and activation of Na,K-ATPase) occur in the state of the plasma membrane of hepatocytes. On the other hand, the changes of the content and

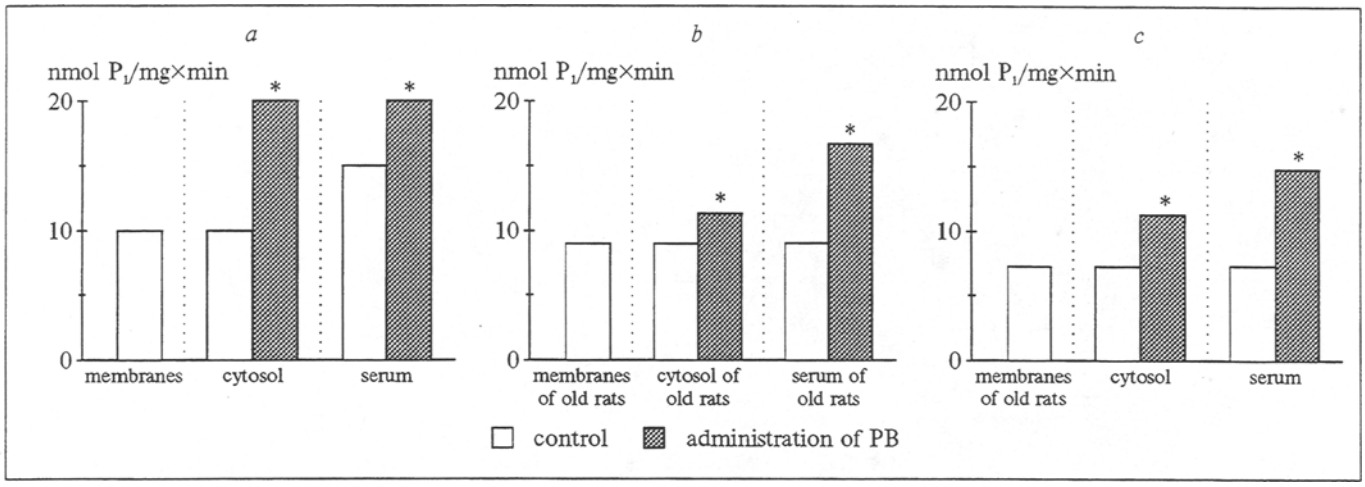

Fig. 2. Effect of hepatocytic cytosol and blood serum of adult (*a*) and old (*b*) rats following PB–caused induction on activity of Na,K–ATPase in fraction of plasma membranes of hepatocytes from rats of various age. *1*, *2*, and *3*) membranes, cytosol, and serum, respectively, of adult rats; *4*, *5*), and 6) membranes, cytosol, and serum, respectively, of old rats. Open bars: control; hatched bars: administration of PB. Other designations as in Fig. 1.

activity of microsomal monooxygenases, as well as the shifts in the state of the plasma membranes are less pronounced in old animals.

In our previous studies we showed that specific regulators of the state of the plasma membrane are synthesized inside the cell during activation of protein biosynthesis. These regulators directly activate Na,K-ATPase in the plasma membrane [8,10,11]. For elucidation of the possible role of these intracellular regulators in age-specific changes in the plasma membrane under conditions of genetic induction of the microsomal oxidation system, we carried out a series of experiments with cell hybrids. These involved adding plasma membranes of intact rat hepatocytes to the cytosols derived from control animals and from animals given PB. As is seen from Fig. 2, *a*, in the case of adult animals the cytosol derived from intact rats failed to cause any changes in the Na,K-ATPase activity. On the other hand, the cytosol derived from the animals following genetic induction of microsomal monooxygenases caused a pronounced increase in the activity of Na,K-ATPase (by 88%). This implies that during genetic induction of monooxygenases, a factor which activates Na,K-ATPase is present in the cytosol of hepatocytes. This factor is released into the blood, since, as is shown in Fig. 2, the serum of animals given PB causes a marked increase in the Na,K-ATPase activity. In old animals this mechanism of intracellular regulation of the state of the plasma membrane is altered. In this case, as in adult animals, the intact plasma membranes do not respond to the action of the control cytosols by changing their level of Na,K-ATPase. Addition of the cytosol derived from the old animals undergoing genetic induction to the fraction of plasma membranes causes a slight but reliable increase of the Na,K-ATPase activity. However, as is seen from Fig. 2, activation of Na,K-ATPase is less pronounced in old rats than in adult animals. The blood serum of old rats undergoing genetic induction also causes activation of Na,K-ATPase in the fraction of plasma membranes (Fig. 2). It seems likely that these age-specific changes in the cytosol are associated not with a change in the state of the plasma membrane, but with changes in the synthesis of invertor per se. The next series of experiments, in which the cytosol of adult animals was exposed to induction by the addition of intact membranes derived from old animals, supports the above assumption. As is seen in Fig. 2, in this case the increase of the Na,K-ATPase activity is the same for old and adult animals. The blood serum of adult animals also activates the enzyme in the membranes derived from old rats. This leads to the conclusion that the ability of the membrane complex to respond to intracellular regulators does not weaken in senescence, but the synthesis of these intracellular regulators is altered with age.

Hence, our findings attest to a definite correlation between the reduced capacity for genetic induction in senescence and the pattern of changes of the state of plasma membranes (MP and the activity of Na,K-ATPase). It seems that in old age the shifts in the state of the plasma membrane are due to the reduced synthesis of intracellular factors of regulation in the cytoplasm. We have previously shown [9] that hyperpolarization of the cell membrane reduces the PB-induced synthesis of microsomal monooxygenases. The changes were more pronounced in old animals than in adults.

Thus, age-related changes in the relationship between the state of the plasma membrane and the rate of microsomal oxidation play an important role in the mechanisms of aging. During genetic induction of microsomal monooxygenases the shifts in the state of the plasma membrane by themselves affect the inductive synthesis.

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