Relationship between Psychological and Bioenergetic Characteristics in Neurotic Old Rats

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Old rats are found to have greatly reduced capacities for learning conditioned responses of various types, particularly when induced to develop a neurosis-like state which impairs autonomic functions and their regulation. The development of hypertensive responses is accompanied at the cellular level by a significant rise of succinate dehydrogenase activity in the sensorimotor cortex of young neurotic rats and by its significant fall in that of their old counterparts, in which the predominant oxidation substrates are NAD-dependent compounds.

Key Words: *neurosis; arterial pressure; Hildebrandt's index; succinate dehydrogenase; NADH dehydrogenase*

Studies carried out during the last 10-15 years have established that at the subcellular level one consequence of acute and chronic forms of stress that result in the development of a neurosis-like state in experimental animals is damage to membranes, primarily those of brain cells, and that at the basis of the damage is the natural mechanism of free-radical oxidation. The free radicals $O_2^{\cdot-}$, $H_2O_2^{\cdot-}$, and $OH^{\cdot}$ are mainly formed in mitochondria in the course of their normal functioning and can cause damage to lipids, proteins, and nucleic acids of the mitochondria themselves. Although the stochastic processes of free-radical damage probably involve only a small proportion of mitochondrial cells at any given time, the lesions they cause in these cells accumulate during ontogeny as well as under stress such as that associated with neurosis [5].

Because mitochondria are the principal sites of energy transformations, the damage they suffer under stress and during aging weakens the energetic power of the cells, and this is reflected in a variety of alterations, notably in physiological responses, resistance of the body to external stimuli, protein synthesis, and membrane permeability [6].

Both aging and stress alter the structural status of membranes by increasing their content of cholesterol and, consequently, the cholesterol/phospholipid ratio in the membranes, i.e., by decreasing their fluidity [2]. Such membranes are more resistant to the injurious action of free radicals but much less capable of performing their intrinsic functions. Here we have a case of pathological adaptation at the molecular level.

The question is, what are the pathogenic events leading to a neurosis-like state in old animals? The present study may be viewed as an attempt to find approaches to how this question can be answered.

MATERIALS AND METHODS

A total of 142 Wistar rats, bred at the Stolbovaya Nursery of the Russian Academy of Medical Sciences, were used, including 102 old rats (aged 18 months) and 40 young adult animals (3-4 months old). Groups of 20 old and 20 young rats were induced to develop a neurosis-like state using the procedure adopted in our laboratory, which involves

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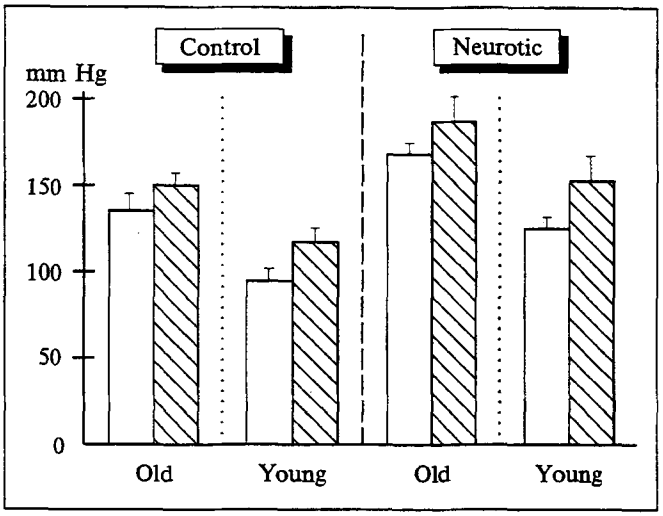

Fig. 1. Arterial pressure in normal and neurotic old and young rats before (white bars) and after (hatched bars) immobilization for 2 h.

daily exposure to white noise (80 dB above the auditory threshold of the human ear) for 4 h per day in combination with sessions of unavoidable painful stimulation of the paws with electric current (2 mA) delivered in a stochastic mode as a reinforcement of rhythmic light flashes (0.5 Hz). Before and one week after these combined exposures, the rats were tested for: 1) behavior in an "open field," 2) the speed at which they learned the conditioned responses of active and passive avoidance, 3) natural and conditioned feeding and drinking reflexes, and 4) the emotional resonance

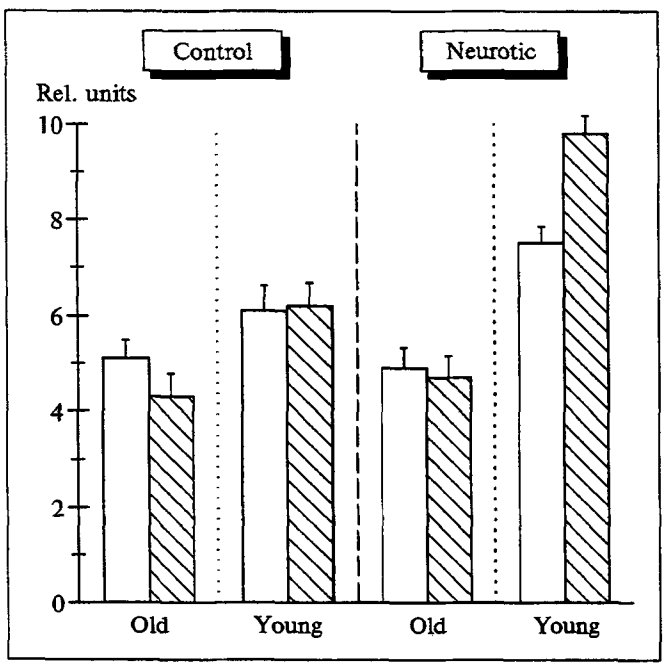

Fig. 2. Hildebrandt's index (heart rate/respiratory rate) in normal and neurotic old and young rats before (white bars) and after (hatched bars) immobilization for 2 h.

reaction. In addition, measurements were made of systolic arterial pressure (on the tail, using photoplethysmography), heart rate, and respiratory rate before (in the resting state) and during functional loading (immobilization for 2 h). One week after the completion of physiological tests, cryostatic sections through the sensorimotor cortex of the brain were prepared and assayed for succinate dehydrogenase (SDH) and NADH dehydrogenase (NADH-DH) activity using nitro blue tetrazolium (as described by Nartsissov *et al.* [7]); enzyme activity was expressed in mg of formazan per μg tissue.

RESULTS

Emotional resonance. When the studied population of rats was first tested at the ages between 2 and 2.5 months, 54% of the rats did not avoid the cry of the victim, 35% avoided it, while the remaining 11% showed ambivalent responses. With advancing age, a significant increase occurred in the proportion of rats remaining in that compartment of the emotional resonance chamber where they had been placed initially rather than choosing to move to another compartment. Thus, 70% of the rats that had avoided the victim's cry and 50% of those that had not when first tested failed to move to another compartment during the testing carried out 18 months later, and the induction of a neurosis-like state as described above had little effect on this ratio. These observations correlated with the behavior of neurotic old rats in the open field, where the levels of their motor and exploratory activity were some 10 times lower than the baseline, although emotional reactions had not been lost.

Conditioned passive avoidance response. Rats learned this response in a standard chamber and were tested for it 7 days later (observation time 180 sec). After a single session, the response persisted for 18 months in 37% of the rats. Among the rats that exhibited this response at that time, the proportion of animals which initially avoided the victim's cry did not differ from that which did not.

Conditioned active avoidance response. Old rats were all incapable of learning this response, but some of them (10%) exhibited an escape response.

Conditioned feeding and drinking responses. Young and old rats did not differ in the rate at which they learned these responses, but the number of eaten sunflower seeds, water-drinking time, and the latent period were significantly greater in the group of old rats.

Arterial blood pressure, heart rate, and respiratory rate. Old normal rats had a higher arterial

pressure both before and after the 2-hour immobilization than younger ones (Fig. 1). Induction of a neurotic-like state elevated blood pressure in both old and young rats, though to a somewhat greater extent in the former. Arterial pressure rise is an adaptive response directed at overcoming oxygen deficiency [9,10].

Calculations of Hildebrandt's index (the ratio of heart rate to respiratory rate) showed that the normal young rats had a significantly higher index than the old ones, both before and after 2-hour immobilization (Fig. 2). Moreover, the immobilization significantly lowered this index in the old rats and virtually did not change it in the young. In contrast, as can be seen in Fig. 2, it had little effect on Hildebrandt's index in the old neurotic rats but significantly raised it in their younger counterparts. This increase mainly occurred as a result of accelerated heart rate, while the respiratory rate decreased only slightly. Hildebrandt's index reflects the extent to which the cardiovascular and respiratory systems function in harmony, and its excessive growth [5] signifies that these systems are no longer interacting in an integrated manner and that there is not enough oxygen, primarily in the brain, to support normal bioenergetic processes in the cells.

The state of bioenergetic processes was assessed by measuring the activity of two respiratory enzymes, SDH and NADH-DH. No histochemical data on the relationship between these enzymes could be found in the available literature.

In old rats both SDH and NADH-DH were found to have lower activity initially than in the young animals (Fig. 3). The production of neurosis led to a significant rise of SDH activity in the young rats - an indication of enhanced oxidative metabolism in the brain. Oxidation of succinic acid is known to inhibit the oxidation of other substrates of the Krebs cycle, and this apparently explains why NADH-DH activity declined in these rats [4]. Succinic acid oxidation occurs at a faster rate and consumes more oxygen than the oxidation of NAD-dependent products, and is often viewed as an emergency biochemical adaptation to so-called peak situations. In other words, the oxidation proceeds by the succinic acid pathway if oxygen is in sufficient supply.

There is a large body of data [1] on how mitochondria change in aging rats. The first mitochondrial structures to undergo alteration are the cristae: these degenerate into formless clumps which then encroach upon the outer mitochondrial membrane. In addition, the mitochondria accumulate lipofuscin as a peculiar adaptive reaction to hypoxia. When mitochondria are damaged, the mechanism of inhibition has been shown [3] to involve inhibition of the succinic acid-oxidizing enzyme SDH by the oxaloacetic acid which is then produced. This mechanism probably underlies the metabolic changes that occurred in the brain of an old neurotic rat: the store of SDH activity in the damaged mitochondria was rapidly depleted so that this activity became inhibited, preventing the mitochondrial membranes from being damaged as a result of the excessively strong stimulation of cellular respiration in our experiments. In stressful situations, oxidative phosphorylation in old rats is bound to rely on the pathway of NAD-dependent substrates, and this possibly accounts, in part at least, for the depressed SDH activity and elevated NADH-DH activity we observed in the neurotic old rats (Fig. 3).

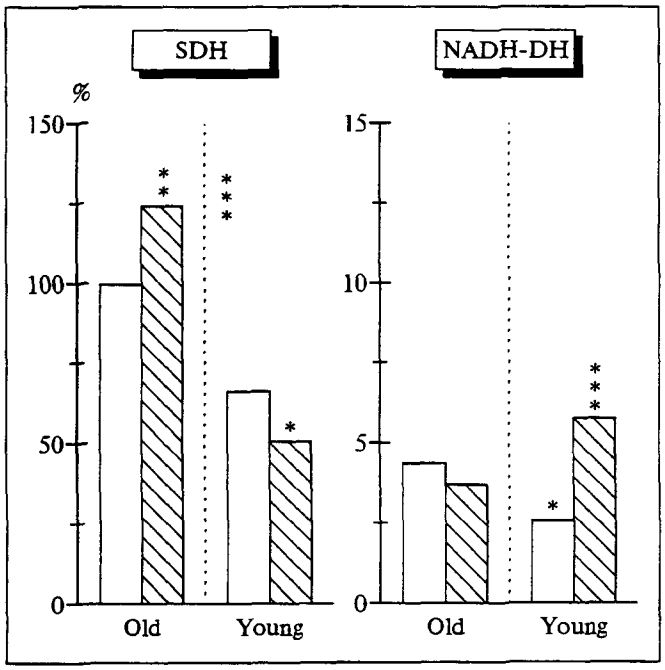

Fig. 3. SDH and NADH–DH activity in normal (white bars) and neurotic (hatched bars) young and old rats. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with normal rats.

Thus, as follows from the above, the emotional resonance reaction and motor and exploratory behaviors in the "open field" were all undergoing extinction in aging rats, and the extinction proceeded at an accelerated pace when old rats were made neurotic. While the conditioned passive avoidance response was retained by 37% of the old rats, the conditioned active avoidance response had been completely extinguished in all such rats and none of them was able to learn it. The progression of hypertension in old rats was not accompanied by a change in the heart rate/respiratory rate ratio, which suggests that the potential for

regulation of the cardiovascular and respiratory systems was exhausted. The old rats also had much lower SDH and NADH-DH activities than did the young. In neurotic young rats, bioenergetic processes in cells of the sensorimotor cortex follow the succinate-dependent pathways, whereas in neurotic old rats the predominant oxidation substrates in these cells are NAD-dependent compounds.

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