Effect of Substance P on Rat Survival after Brain Ischemia: Effect Depends on Behavior Types

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It was established in our previous studies that the survival of rats with circulatory brain hypoxia depends on the types of behavior and on the expression of structural metabolic changes (mitochondrial hypertrophy, an increase of succinate dehydrogenase activity) developing in the brain soon after ischemia [3]. Since these structural changes may be regarded as timely adaptive reactions [1,5] of the brain tissue to hypoxic stress [6], it would seem to be possible to alter resistance to circulatory brain hypoxia by changing the degree of expression of these reactions.

For this purpose we used substance P (SP), a neuropeptide with an antistressor (adaptogenic) effect. This effect is known to be shown under conditions of both acute [8] and chronic [4,7] emotional stress and to depend on individual typological pecularities of animal behavior [8].

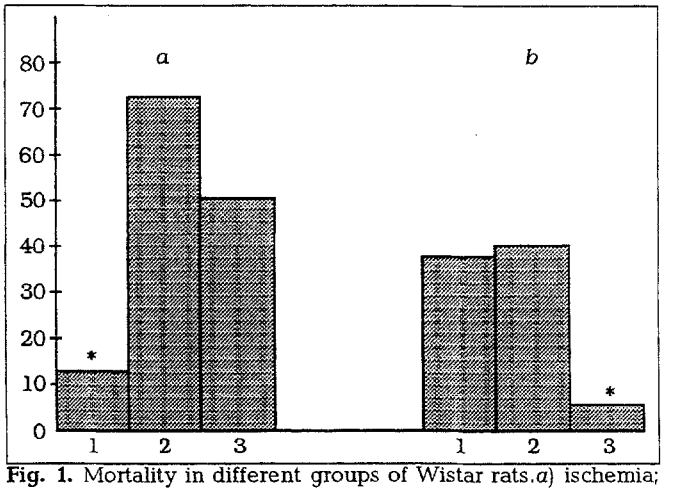

Fig. 1. Mortality in different groups of Wistar rats. *a*) ischemia; *b*) ischemia with SP injection; 1) rats with active, 2) rats with passive behavior; 3) middle group. Numerical data show mortality (%); asterisk: differences in mortality are reliable ($p<0.05$) as compared to middle group; n: number of animals in group.

MATERIALS AND METHODS

Experiments were carried out on 129 Wistar rats and 202 nonpedigree rats (males). The type of behavior of the animals was determined in "open field" and "forced swimming" tests. The technique was described in detail previously [3]. The animals were divided into 9 groups: two extreme groups, one with an active behavior type (high motor activity and a low level of depression) and one with a passive behavior type (low motor activity and a high level of depression), one middle group (median behavior indexes), and 6 mixed groups. Brain ischemia was induced by bilateral ligation of the carotid arteries. In 55 Wistar rats survival was determined 48 h after

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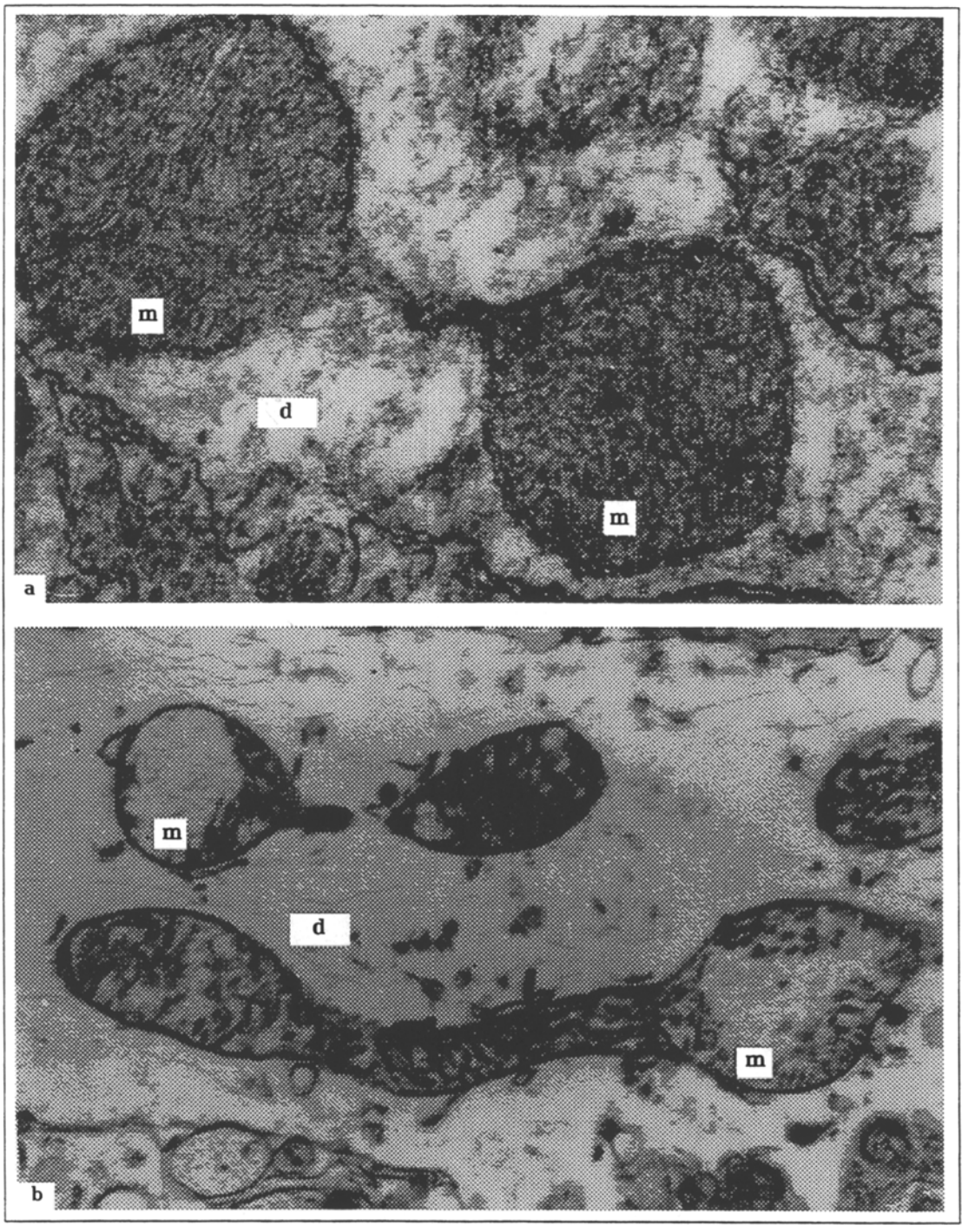

Fig. 2. Hypertrophy and division of mitochondria in dendrite of sensorimotor cortex in a rat with active behavior type. *a*) 1 h after brain ischemia, ×70,000; *b*) 1 h after ischemia with SP injection, ×50,000; m: mitochondrion, d: dendrite.

brain ischemia without SP injection, and in 59 rats with SP injection. Twelve rats from the middle group and the two extreme groups (4 rats from each group) without ischemia were used as the control to determine the initial succinate dehydrogenase activity (SDH).

SDH activity was tested by the quantitative histochemical method [2]. The enzyme activity was expressed in conventional units (c.u.) - mmoles of formazan per mole of protein nitrogen in 1 min. In 92 nonpedigree rats (from the middle and two extreme groups) killed 1,24, and 48 h after brain ischemia both with and without SP the microstructural (electronic microscopy of the sensorimotor cortex) and metabolic (SDH activity in the sensorimotor cortex) changes of the brain were examined. SP in a dose of 250 μg/kg was injected 30 min after carotid

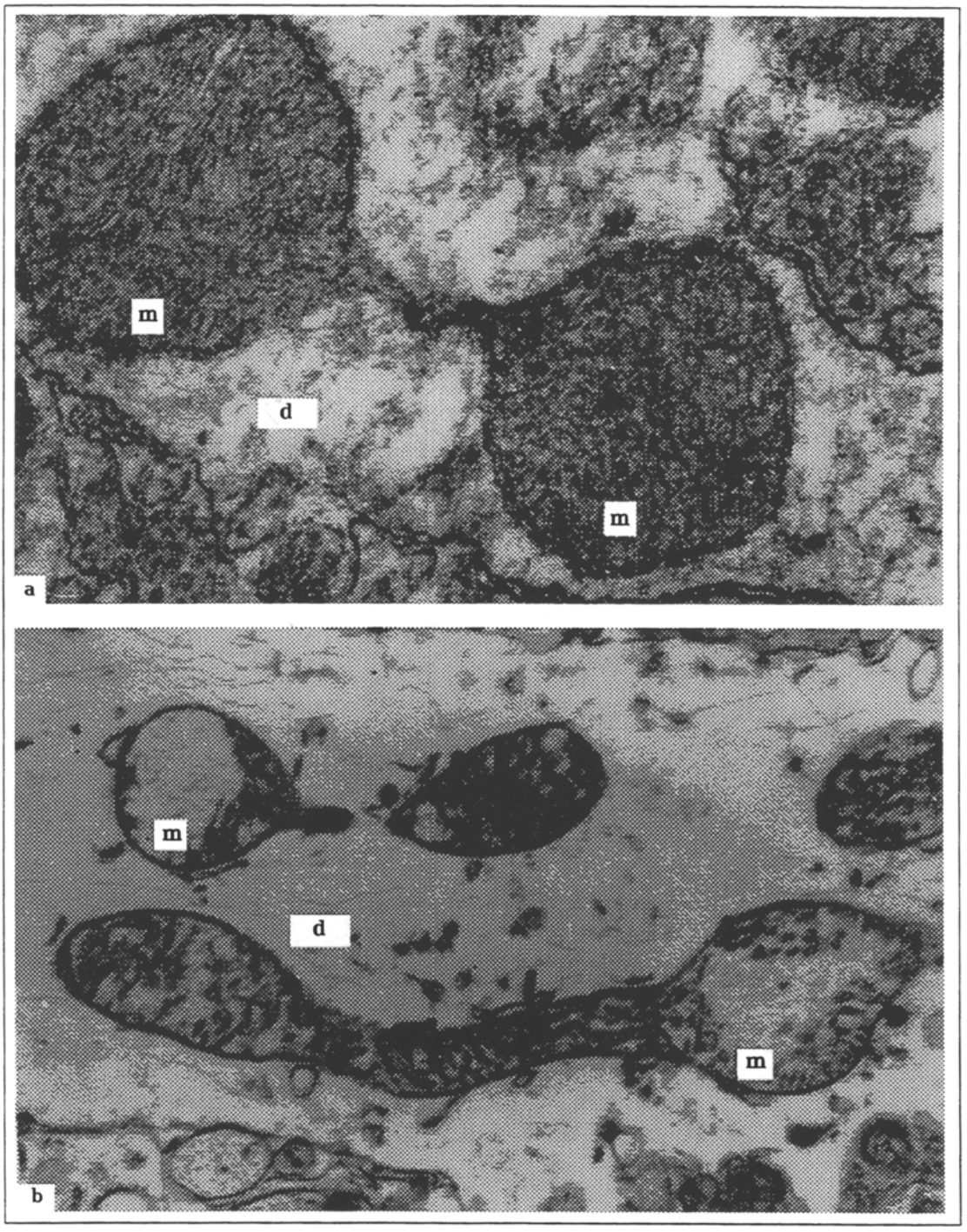

Fig. 3. Destruction of hypertrophied mitochondria in neuron cytoplasm. *a*) rat with active behavior type 1 h after brain ischemia with SP injection; arrow shows site of total destruction of granular reticulum; r: ribosomes; cyt: cytoplasm; ×60,000; *b*) rat with passive behavior type 1 h after ischemia; n: nucleus; ×60,000.

artery ligation. The results obtained were processed statistically on a PC/AT using developed software.

RESULTS

Ischemia with SP injection caused the death of 27 out of 55 Wistar rats. From the data obtained the total mortality level (49%) in Wistar rats was found to be reliably higher as compared to the nonpedigree rats (29%, x^2_1=4.35 with the Yates's correction for continuity, p=0.037). In Wistar rats with the active behavior type the mortality level (12%) was found to be reliably lower (x^2_1=7.7, p=0.005) than in rats of the middle group (73%) (Fig. 1, *a*). Therefore, in

Wistar rats as well as in nonpedigree rats [3] the maximum mortality was found in the middle group and the minimum mortality in rats with the active behavior type. A tendency (x^2_1=2.71, p=0.1) was observed toward a decrease of the total mortality after ischemia with SP injection (49% without injection, 84% with injection). At the same time, rats with active behavior survived for the most part after ischemia without SP (Fig. 1, *a*), whereas, on the contrary, rats with passive behavior survived predominantly after ischemia with SP (Fig. 1, *b*). SP unreliably raised the mortality in rats with the active behavior type (x^2_1=2.64, p=0.2), unreliably lowered it (x^2_1=2.01, p=0.15) in rats of the middle group, and reliably lowered it in rats with the passive behavior type (p=0.04). SP was found to reduce the reactive changes in the mitochondria (hypertrophy and division) developing in the brain 1 h after ischemia. The effect was most pronounced in rats with active behavior (Fig. 2). Whereas 1 h after ischemia without SP the destructive changes in the mitochondria were expressed markedly less in rats with active behavior, in the case of ischemia with SP the destructive processes in the mitochondria in rats with active behavior were similar to those in rats with passive behavior 1 h after ischemia without SP (Fig. 3). In some cases, in rats with the active behavior type more severe pathological changes were observed in the brain 1 h after ischemia with SP injection as compared to ischemia without SP, including granular reticulum degradation, vacuolization of cytoplasmic canals, degeneration of mitochondria (both hypertrophied and unenlarged). These changes were found in neurons and in glial cells. Locally, associations of small (nonhypertrophied) mitochondria were detected in the cell cytoplasma and cell processes. Destructive changes in the mitochondria in rats with the active behavior type 1 h after ischemia with SP injection were similar to those in rats of the same group 48 h after ischemia without SP. In other words, ischemia with SP led to earlier destructive changes in the brain than ischemia without SP. As a result of the increased mortality in rats with the active behavior type after ischemia with SP, and, consequently, the survival of more resistant animals, the latter show a better preservation of the mitochondria 48 h after ischemia with SP that rats of the same group 48 h after ischemia without SP.

Conversely, the decrease of the post-ischemia mortality due to SP in rats with passive behavior resulted in the survival of individuals with more severe brain pathology. Consequently, in rats with the passive behavior type 48 h after ischemia with SP significant changes in the brain were detected similar to those in rats with the active behavior type 48 h after ischemia without SP. Therefore, the greater the adaptive changes in the mitochondria soon after ischemia, the lower the mortality, and the more severe the destructive changes in the brain of surviving animals in the long term after ischemia - "the structural price of adaptation" (Meerson) [1].

One hour after ischemia without SP a significant acceleration of the oxidative metabolism of the brain was found, more pronounced in rats with active behavior (SDH activity rose to 170.5±6.7; in the control 127.5±0.4 c.u.), than in rats with passive behavior (SDH activity rose to 130.8±0.7; in the control 102.2±7 c.u.) and in rats of the middle group (SDH activity rose to 133.0±1.6; in the control 116.2±0.3 c.u.).

One hour after ischemia with SP the SDH activity increased to a lesser extent: in rats with active behavior to 161.0±2.0, in rats with passive behavior to 115.2±1.0 and in rats of the middle group to 121.1±3.4 c.u.

The results suggest that the effect of SP on the resistance to circulatory brain hypoxia depends on the type of behavior: it raises resistance in rats with passive behavior, lowers it in rats with active behavior, and does not affect it in rats of the middle group.

The data point out the necessity of individualizing the approach to peptide use for the purpose of increasing the resistance to brain ischemia and open up new prospects for research into remedies with a differential therapeutic effect on individuals of different types.

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