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Effects of Ischemia on the Expression of Neurotrophins and Their Receptors in Rat Brain Structures Outside the Lesion Site, Including on the Opposite Hemisphere

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Abstract—Neurotrophins stimulate the regeneration of neural tissue after lesions. It is also known that the sources of neurogenesis and cerebral function recovery are predominantly located in subcortical brain structures. The effects of ischemia on the expression of genes that encode neurotrophins (*Bdnf*, *Ngf*, *Nt-3*) and their receptors (*TrkB*, *TrkA*, *TrkC*, *p75*) in brain structures outside the lesion site were studied 3, 24, and 72 h after irreversible unilateral occlusion of the middle cerebral artery in rats. Changes in the mRNA expression of these genes were assessed by relative quantification using real-time RT-PCR. Sham surgery was found to stimulate the expression of genes that encode neurotrophins (*Bdnf*, *Ngf*) and their receptor (*p75*). It has been shown that ischemia influenced the expression of neurotrophins (*Bdnf*, *Ngf*, *Nt-3*) and their receptors (*TrkB*, *TrkA*, *TrkC*, *p75*) in brain structures outside the lesion focus, including the contralateral hemisphere. The downregulation of *Bdnf* and *TrkB* transcripts and *Ngf* and *TrkA* upregulation in the contralateral cortex on the first day of ischemia obviously reflected stress response. On day 3, *Nt-3* transcription increased in all investigated structures outside the lesion focus. In the contralateral hemisphere, relative levels of *TrkA* and *TrkC* mRNA expression increased, while *p75* expression decreased. Presumably, the observed changes in gene transcription serve to facilitate neuroplasticity and neural tissue regeneration.

Keywords: BDNF, NGF, Nt-3, TrkA, TrkB, TrkC, p75, real-time qRT-PCR, Wistar rat, focal cerebral ischemia, mRNA expression, neuroplasticity

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INTRODUCTION

Neural plasticity, a phenomenon first described by neurologists, consists of compensation for CNS functions following massive brain lesions. Apparently, neuroplasticity is based on the brain's ability to form new synaptic connections, as well as on neoneurogenesis. In the course of individual development, neurotrophins activate proliferation and the differentiation of neural progenitor cells; at the same time, in a mature organism, similar to other trophic factors, neurotrophins can act to block damage and stimulate neural regeneration in different pathological neurological conditions [1–5]. In an adult organism, neurogenesis is supported by layers of poorly differentiated cells located by the walls of the lateral ventricles and in the dentate gyrus of the hippocampus [6]. Trophic factors, such as Egf and Fgf2, stimulate the proliferation of subventricular zone cells of the lateral ventricles in the mouse [7]. Experiments with focal (local) brain ischemia demonstrated the activation of neuro- and angiogenesis in the subventricular zone [8, 9]. It was also shown that neural progenitor cells of the subventricular zone migrated towards Egf and Tgf-α in the mouse in vivo [10, 11]. The phenomenon of neuroplasticity in brain pathology is based on the stimulation of the proliferation of neural progenitor cells, which are located in subcortical structures and serve as neurogenesis reservoirs, as well as on their subsequent migration for structural and functional regeneration of damaged brain regions.

On the other hand, cortical spreading depression, or spreading depolarization (CSD), which contributes to the formation and expansion of the lesion in brain ischemia, can stimulate the transcription of *Bdnf* and *TrkB,* which encode brain-derived neurotrophic factor and its receptor, respectively, in the rat cortex [12]. Apparently, depression induced by an increase in the extracellular K^+ concentration in the necrotic focus affects not only the brain area surrounding the focus (penumbra), but also the subcortical structures: the

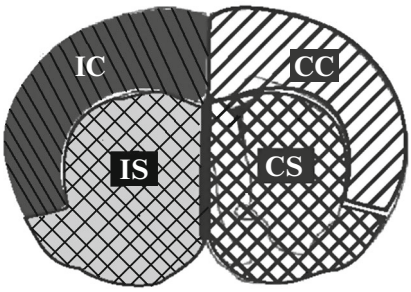

Fig. 1. Map of regions of the rat brain where changes in the transcription of neurotrophin and neurotrophin receptor genes were determined after irreversible occlusion of the left middle cerebral artery. IC is the frontoparietal cortex of the ipsilateral hemisphere, where the focus of ischemic damage was located; CC is the frontoparietal cortex of the contralateral hemisphere; IS is the subcortex of the ipsilateral hemisphere; CS is the subcortex of the contralateral hemisphere.

hippocampus and the thalamus [13–16]. It has been demonstrated experimentally that neurotrophins participate in the structural and functional regeneration of nerve tissue after ischemic damage [2, 17–19]. Local and parenteral administration of trophic factors (Bdnf, Igf-1, Gdnf, Vegf) helped to diminish the edema and size of the brain lesion in focal ischemia [2–5, 20, 21]. Thus, neurogenesis reservoirs are located predominantly in subcortical brain structures and seem to be stimulated by neurotrophins. We previously investigated the effects of ischemia on the expression of neurotrophins and their receptors in the cortical lesion induced by focal brain ischemia in rats [22]. Cerebral ischemia activated the transcription of neurotrophin genes in the brain cortex, including the lesion, but at the same time, it reduced the transcription of genes that encode their receptors, which apparently reflected the insufficiency of the cells' survival potential. Based on these results, as well as on the data that suggest the potential involvement of neurotrophins expressed in adjacent, remote, and neurogenic regions of the brain in the compensation of CNS functions after damage, we analyzed the effects of ischemia on the expression of genes that encode neurotrophins and their receptors in the brain of experimental animals outside the lesion site, in particular in the subcortical structures of both hemispheres and in the contralateral cortex.

EXPERIMENTAL

Experimental model of focal brain ischemia. The study was performed in 2-month-old male Wistar rats weighing 270–320 g (n = 47), who were kept under natural light conditions and with food and water supplied ad libitum. Each experimental group included five to seven animals, except for the "72 h with ischemia" group, which comprised four rats. Focal brain ischemia was induced by coagulative occlusion of the distal left middle cerebral artery (upstream of the *a. lenticulostriatis* branch) [23]. The surgery was performed under anesthesia with chloral hydrate (300 mg/kg intraperitoneally). For each time point (3, 24, and 72 h after surgery), a group of sham-operated rats was used as a positive control. Control animals were subjected to a similar procedure of surgical site preparation under the same anesthesia, but no artery occlusion was performed. After 3, 24, or 72 h, the rats were decapitated using short-term ether anesthesia, which has a minimal effect on metabolic processes [24, 25]. Specimens of the frontoparietal cortex and the corresponding complex of subcortical structures, including the thalamus, basal nuclei, walls of the lateral ventricles, and the hippocampus, were isolated. In rats with occlusion, structures of both brain hemispheres were analyzed: the frontoparietal cortex of the right (contralateral) hemisphere (CC), subcortex of both hemispheres (IS and CS), as well as the frontoparietal cortex of the left (ipsilateral) hemisphere (IC), where the zone of ischemic damage was located, according to the previously published histological data (Fig. 1) [22, 26]. Tissues were frozen in liquid nitrogen and stored at –70°C.

RNA isolation and cDNA synthesis. Total RNA from rat brain tissues was isolated using the conventional guanidine-thiocyanate procedure [27]. The obtained RNA was treated with DNAse I (MBI Fermentas, Lithuania) in the presence of an RNase inhibitor as recommended by the manufacturer. Proteins were removed by phenol–chloroform extraction (1 : 1). RNA was quantified by spectrophotometry [28], and its quality was verified by electrophoresis in agarose gel under denaturing conditions. All specimens were found to contain clear 28S and 18S bands with a ratio of intensities close to 2 : 1. cDNA was synthesized using a RevertAid First Strand cDNA Synthesis kit as recommended by MBI Fermentas in a reaction volume of 20 μL containing 5 μg RNA.

Real-time RT-PCR. Primer specific to genes that encode neurotrophins and their receptors were designed with the OLIGO Primer Analysis Software 6.31 and synthesized by Syntol (Russia) [22, 29]. The specificity of primers that should give rise to a unique product of cDNA amplification was verified by electrophoresis of PCR products. PCR reaction mixture (25.5 μL) contained a 0.02-μL aliquot of the reverse transcription product, 6 pmol of either forward and reverse primers to the target gene and to *Gapdh*, which is consistently expressed in experimental ischemia [30, 31] and was used as the internal reference, and 10 μL of 2.5× reaction mixture (Syntol), which contained 2.5× PCR buffer, *Taq* DNA polymerase, dNTPs, glycerol, Tween 20, and SYBR Green I intercalating dye. Specimens of cDNA derived from brain tissues were amplified in an Mx3000P system for real-time PCR (Stratagene, United States) using the following protocol: segment 1 consisted of 2 min at 50°C; segment 2 (denaturation) consisted of 10 min at 95°C; segment 3

(amplification with a single fluorescence measurement) consisted of 40 cycles of 30 s at 95°C, 30 s at 64°C, and 30 s at 72°C; and segment 4 (melting) consisted of 1 min at 95°C, 30 s at 64°C, and 30 s at 95°C. Each cDNA specimen was analyzed in three replicates. The efficiency of amplification with each primer pair and the threshold fluorescence level (Ct) were calculated using the Mx 3000p v2.00 software.

Statistical analysis. Data on the expression levels of the target and the reference gene mRNAs were analyzed using the randomization test (differences were considered significant at $P \le 0.05$) with the Relative Expression Software Tool (REST©), which determines the significance of differences between groups by expression levels of a target gene normalized to expression levels of the internal reference gene [32, 33]. The relative expression level (R) was calculated as follows: $R = (E_{target})\Delta Ct(_{target})/(E_{ref})\Delta Ct(_{ref})$, where E_{target} is the efficiency of PCR for the target gene, E_{ref} is the efficiency of PCR for the reference gene, $\Delta Ct(_{target})$ is the difference between mean Ct values for the target gene in the control and the experimental groups, and $\Delta Ct(_{ref})$ is the difference between mean Ct values of the reference gene in the control and the experimental group. Relative transcript levels in the brain structures of sham-operated rats and of animals with focal ischemia were normalized to the levels of the corresponding transcripts in the same tissues of intact animals, which were taken for 100% (Fig. 2) and compared to the levels in sham-operated and intact animals.

RESULTS

We determined the relative transcription levels of genes that encode neurotrophins (*Bdnf*, *Ngf*, and *Nt-3*) and their receptors (*TrkB*, *TrkA*, *TrkC*, and *p75*) in the frontoparietal cortex and in subcortical structures, including the thalamus, the basal nuclei, the walls of the lateral ventricles, and the hippocampus, in both brain hemispheres of rats subjected to unilateral irreversible occlusion of the middle cerebral artery, as well as in the same structures of sham-operated animals in 3, 24, and 72 h after surgery (Fig. 2). In 3 h post-occlusion, the expression of *Bdnf* transcripts in the contralateral hemisphere of experimental animals (CC and CS) decreased by more than twofold compared to their levels in the cortex and subcortex of sham-operated animals (Fig. 2a); moreover, the level of *Bdnf* mRNA in the contralateral hemisphere (CC and CS) was significantly lower than in the ipsilateral one. In the cortex of sham-operated rats (LC) and in the ipsilateral cortex of ischemic rats (IC), the relative levels of *Bdnf* transcripts were significantly lower than in intact animals. In 24 h, *Bdnf* transcription in the ischemic focus increased significantly, but did not change outside the lesion site. In 72 h, *Bdnf* expression in sham-operated rats (LS) was higher than in intact animals. At the same time, we observed a decrease in relative *Bdnf* mRNA levels in the subcortex of both hemispheres (IS, CS) of animals with ischemia compared to sham-operated ones. A similar trend to downregulation of *Bdnf* expression was observed in the contralateral frontoparietal cortex (CC), in contrast to an insignificant increase in its expression in sham-operated animals (LC) (Fig. 2a). In our model, we observed a decrease in the levels of *TrkB* transcripts, which encode Bdnf receptors, in the contralateral cortex (CC) in 3 h and in the subcortex of the ipsilateral hemisphere (IS) in 72 h after occlusion (Fig. 2a).

Figure 2a also presents the data on the expression of *Ngf* and *TrkA* mRNAs, which encode nerve growth factor and its high-affinity receptor, respectively. Three hours after occlusion, the levels of *Ngf* mRNA in the contralateral cortex (CC) and in the cortex area including the ischemic focus (IC) were increased in comparison to their levels in the cortex of sham-operated animals (LC). In 72 h, in the subcortex of sham-operated rats (LS), the level of *Ngf* mRNA was increased in comparison to intact animals, similarly to what was observed for *Bdnf* expression. In contrast to this increase, the abundance of *Ngf* transcripts in the subcortex of both brain hemispheres of rats with occlusion (IS and CS) was significantly lower than in sham-operated rats (LS). Twenty-four hours after occlusion, we observed an increase in *TrkA* expression both in the ischemic (IC) and in the contralateral cortex (CC) compared to sham-operated (LC) and intact rats. Seventy-two hours after occlusion, the level of *TrkA* mRNAs in the subcortex of the contralateral hemisphere (CS) of animals with ischemia was higher than in sham-operated rats (LS) (Fig. 2a). The relative levels of *TrkA* transcripts determined in the subcortex (IS, CS) of rats with ischemia (in 3 h) and in the cortex of sham-operated rats (LC, in 72 h) were decreased compared to intact animals.

Three hours after occlusion, the relative *Nt-3* mRNA levels in the contralateral cortex of animals with ischemia (CC) were significantly higher than in sham-operated rats (LC) (Fig. 2b); they were also higher than in the ipsilateral cortex including the ischemic focus (IC). In 24 h, the levels of *Nt-3* transcripts were increased in the subcortex of both hemispheres (IS, CS); however, the difference was significant only in comparison to intact animals. After 72 h, the relative levels of *Nt-3* transcripts in the contralateral cortex (CC) and the subcortex of both hemispheres (IS and CS), as well as in the ischemic ipsilateral cortex (IC) were higher than in sham-operated rats. In our experiments, changes in *TrkC* expression in brain areas outside the ischemic focus were observed 72 h after surgery; it was decreased in the subcortex of the ipsilateral hemisphere (IS) and increased in the contralateral cortex (Fig. 2b).

After 72 h, the level of *p75* mRNAs, which encode the low-affinity receptor of all neurotrophins, was lower in the contralateral cortex (CC) of rats after sur-

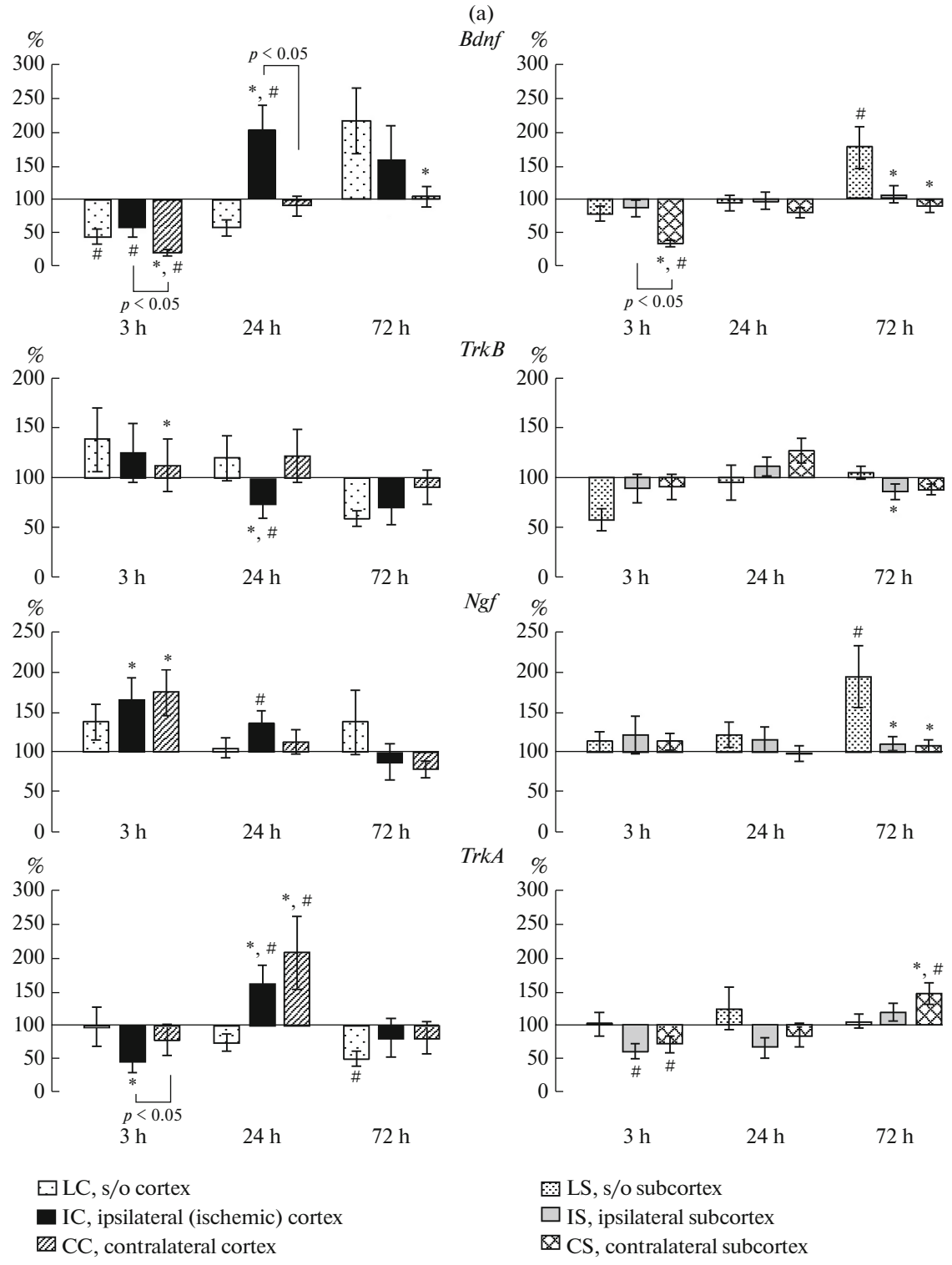

Fig. 2. Changes in the relative levels of neurotrophin and neurotrophin receptor mRNAs in the brain of rats with focal ischemia in 3, 24, and 72 h after the artery occlusion. (a) *Bdnf* and *TrkB*, *Ngf* and *TrkA*; (b) *Nt-3* and *TrkC*, *p75*. S/o is sham-operated; LC is the frontoparietal cortex of sham-operated rats; IC is the frontoparietal cortex of the ipsilateral hemisphere of rats with artery occlusion; CC is the frontoparietal cortex of the contralateral hemisphere of rats with artery occlusion; LS is the subcortex of sham-operated rats; IS is the subcortex of the ipsilateral hemisphere of rats with artery occlusion (ischemia); CS is the subcortex of the contralateral hemisphere of rats with artery occlusion. mRNA levels of target genes were normalized to *Gapdh* mRNA levels used as the internal reference. Data are presented as percentages of the relative expression level of the corresponding gene in intact animals taken for 100% (mean ± S.E). Significant differences ($p < 0.05$): * indicates compared to the corresponding time-point group of sham-operated animals; # indicates compared to the corresponding time-point group of intact animals.

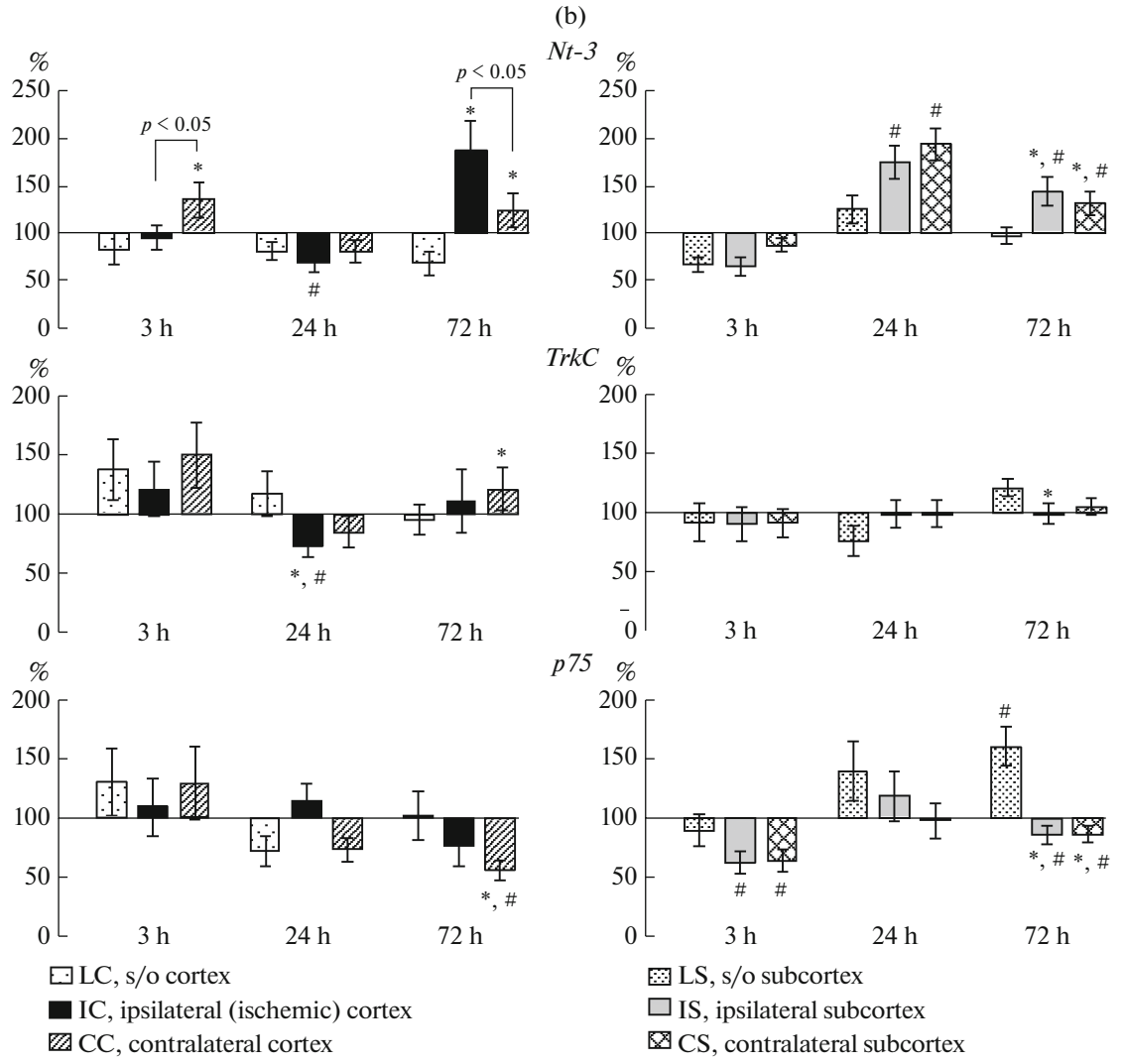

Fig. 2. (Contd.).

gery than in sham-operated (LC) and intact rats (Fig. 2b). In 3 h post-occlusion, the level of *p75* transcripts in the subcortex of both brain hemispheres (IS and CS) was lower than in intact animals, and in 72 h it was lower than both in sham-operated (LS) and in intact rats. In 72 h, the level of *p75* mRNA in sham-operated rats (LS) was higher than in intact animals.

DISCUSSION

Experimental models of cerebral ischemia are employed when studying damaging mechanisms of ischemic stroke, as well as in the development of neuroprotective drugs. An analysis of the expression of genes that encode neurotrophic factors represents an approach that enables the comparison of genomic functioning patterns under normal and ischemic conditions. In these experiments, the most commonly used positive control is sham-operated animals subjected to the preparation of the surgical site, but not to bloodstream occlusion; in some cases, the contralateral hemisphere serves as a control. In our previous work, we investigated the effects of ischemic damage on the expression of genes that encode neurotrophins and their receptors in the brain region comprising the lesion site and the penumbra [22]. Taking into account that stroke-induced local brain damage is accompanied by cortical spreading depression and neuroplasticity effects, as well as in order to determine whether the contralateral brain hemisphere represents an adequate control site in such research, it seems informative to analyze the expression of neurotrophin and neurotrophin receptor genes outside the lesion site, in particular in the unaffected hemisphere.

Our results indicate that, in animals with occlusion of the middle cerebral artery, expression of genes that encode neurotrophins and their receptors was altered not only in the ischemic focus, but also in all other

brain structures studied, i.e., in the frontoparietal cortex of the contralateral hemisphere and in the subcortex of both hemispheres. At the same time, the levels of mRNAs that encode neurotrophins (*Bdnf*, *Ngf*) and their receptors (*TrkA*, *p75*) in sham-operated animals were also different from their levels in the corresponding brain structures of intact rats. For instance, 3 h after surgery, *Bdnf* expression was decreased in the ipsilateral and the contralateral cortex of animals with artery occlusion, as well as in the cortex of sham-operated animals compared to intact rats (Fig. 2*a*). On the one hand, the decrease observed in the contralateral cortex may indicate the reaction of brain regions remote from the focus of ischemic damage. However, taking into account that *Bdnf* expression was also significantly decreased in the brain of sham-operated rats and that at the subsequent time points it was restored to the levels observed in intact animals, we suppose that the early decrease in *Bdnf* expression was related to stress associated with surgery. This notion is further substantiated by data showing the nearly immediate (1 h) downregulation of Bdnf expression in the hippocampus in response to immobilization stress [34]. At the same time, no significant changes in *Bdnf* mRNA expression were observed in experiments with swimming-induced stress [35]. At any rate, we doubt that the decrease in *Bdnf* expression observed in the first hours after occlusion or sham operation is related to the phenomenon of spreading depolarization, which can stimulate neurotrophin production. Indeed, an experiment with induced spreading depression showed that, in 2 h, *Bdnf* and *TrkB* mRNA expression was increased in the affected region of the cortex, but not in the contralateral cortex or other brain structures [12].

Three hours after surgery, the expression of *Ngf* and *Nt-3* in the contralateral cortex of animals with occlusion (CC) was higher than in the cortex of sham-operated rats (LC) (Figs. 2a, 2b). The upregulation of *Ngf* transcription in the contralateral cortex of operated rats in 3 h after occlusion has already been reported previously [36]. In our experiments, no significant effect of occlusion on *Bdnf* and *Ngf* expression in brain regions outside the damaged area was observed in the first 24 h; however, in 72 h, we observed a decrease in *Bdnf* and *Ngf* transcription in the subcortex (IS, CS) of animals with ischemia, while in sham-operated rats (LS), the levels of these mRNAs were significantly increased (Fig. 2a). Thus, in 3 days after the sham operation, *Bdnf* and *Ngf* transcription in the brain of these animals was enhanced. The causes of this are not quite clear; they might be related to post-stress effects, which involve neuroplasticity activation. Nevertheless, the described phenomenon effect raises a question whether experiments of this kind can be performed using sham-operated control only.

According to the data from previous publications, ischemia either did not affect *Nt-3* expression, or decreased the levels of *Nt-3* mRNA and its protein product in the hippocampus [37–39]. It was also found that immobilization stress enhanced *Nt-3* expression in the hippocampus [34]. In our experiments, we observed an increase in *Nt-3* mRNA levels in the ischemic cortex in 72 h after occlusion; moreover, it was shown that, compared to sham-operated animals, *Nt-3* expression was increased in the contralateral cortex (CC) after 3 and 72 h, as well as in the subcortex of both hemispheres (IS, CS) in 24 and 72 h. Taking into account that Nt-3 regulates the formation of new synaptic contacts and neuroplasticity in the hippocampus, the described upregulation of its expression in brain tissues outside the lesion site, in particular in subcortical structures, including the walls of the lateral ventricles, may indicate the beginning of regeneration of neural connections and functions in 72 h after occlusion [40].

It is well known that the effects of neurotrophins are mediated by their interaction with receptors. Indeed, in a rat model similar to ours, it was shown that treadmill exercise activated neuroplasticity, which involved enhanced expression of Bdnf and its TrkB receptor observed not only in the penumbra, but also in the contralateral cortex, as well as in the cortex of sham-operated animals [41]. In some studies on experimental cerebral ischemia, the activation of *TrkB* mRNA expression was observed in the contralateral hemisphere, in particular in the hippocampus; however, in our model, the levels of *TrkB* transcripts were found to decrease in the contralateral cortex (CC) in 3 h after occlusion and in the subcortex of the ipsilateral hemisphere (IS) in 72 h [42–45]. Thus, in our model, transcriptional downregulation of *Bdnf*, a neurotrophin-encoding gene observed in brain tissues outside the lesion site was accompanied by the decreased transcription of *TrkB*, which encodes the Bdnf receptor. This expression pattern observed outside the lesion site apparently reflects the fact that cells processes regulated by Bdnf interaction with TrkB are activated insufficiently.

The levels of *TrkA* transcripts, which encode Ngf receptors, were found to increase in the contralateral cortex (CC) in 24 h after occlusion and in the subcortex of the contralateral hemisphere (CS) in 72 h (Fig. 2a). Our data suggesting that *TrkA* transcription is upregulated outside the ischemic focus agree with the results reported by Chung et al., who showed that physical exercise stimulated TrkA expression in the contralateral hemisphere of animals with brain ischemia [46]. Apparently, an increase in the *TrkA* mRNA level is involved in the activation of neuroplasticity.

The effect of ischemia on *TrkC* expression in unaffected brain regions in 72 h after occlusion was heterogeneous: it was found to decrease in the subcortex of the ipsilateral hemisphere (IS) and to increase in the contralateral cortex (CC) (Fig. 2b).

It should be noted that the effects of ischemia on gene expression outside the lesion site were the most pronounced 72 h after occlusion when, along with

brain-damaging processes, regeneration processes are also activated. At this moment, the levels of *p75* mRNA decrease in the contralateral cortex (CC), as well as in the subcortex of both hemispheres (IS and CS) (Fig. 2b). Along with Trk receptors, the low-affinity p75 neurotrophin receptor plays a controversial role in the regulation of neuron fate; it ranges from potentiating the effects of Trk receptors aimed at neuron survival and differentiation to apoptosis [47]. Apparently, p75 can act to generate optimal neurotrophin concentrations and to stimulate their interaction with Trk receptors [48]. On the other hand, a substantial body of data indicates that p75 expression is involved in neuron death. For instance, increased p75 levels were observed in the ischemic dentate gyrus of the hippocampus and in other neurodegenerative conditions, including multiple sclerosis and Alzheimer's disease [49, 50]. Thus, the observed downregulation of *p75* transcription in the contralateral hemisphere accompanied by an increase in the levels of *TrkA* and *TrkC* mRNAs in the same structures probably suggests the activation of nerve-cell survival and may represent one of the phenomena underlying neuroplasticity. On the other hand, the fact that the levels of *p75, Bdnf,* and *Ngf* transcripts were increased in the subcortex of sham-operated rats in 72 h after sham surgery, while the levels of the corresponding Trk receptor transcripts remained unchanged invites the hypothesis that neuron apoptosis might have been activated in these animals.

Thus, the expression of genes that encode neurotrophins and their receptors was changed in all analyzed structures outside the lesion site. The decrease in *Bdnf* and *TrkB* transcript levels and the increase in *Ngf* and *TrkA* mRNA levels observed in the contralateral cortex in the first 24 h after surgery is likely to reflect the stress response. On the other hand, the upregulation of *Nt-3* transcription observed in 72 h in all structures outside the ischemic focus, in particular in the subcortex including neurogenic zones of the hippocampus and the lateral ventricles can probably be interpreted as contributing to neuroplasticity. It seems that the decrease in the level of p75 transcripts accompanied by upregulation of *TrkA* and *TrkC* expression observed in the contralateral hemisphere in 72 h may also be a sign of neuroplasticity. It should be noted that the sham operation was shown to have a stimulating effect on the expression of *Bdnf*, *Ngf,* and *p75*, which encode neurotrophic factors and their low-affinity receptor, respectively, in the subcortical brain structures. The mechanisms underlying the observed changes in the expression of the genes in question in brain tissues outside the lesion site are currently unclear and require investigation. However, it can be speculated that there exist some mechanisms that serve in the activation of gene expression at significant distances within brain tissues.

We should also point out that the contralateral hemisphere represents an inadequate control for the analysis of gene expression in the models of experimental focal brain ischemia. For instance, it was employed in some studies that investgate the expression of trophic factors (Bdnf, Vegf) in the brains of rats with transitory focal ischemia [51, 52]. Our doubts concerning the validity of this approach to CNS studies are based not only on our own results, but also on the data obtained in the model of photochemically induced unilateral damage of the primary motor cortex [53, 54]. Altered gene expression was observed not only in the lesion focus and the adjacent somatosensory zone of the rat brain (20 genes), but also in the contralateral hemisphere; it involved eight genes in the zone corresponding to the damage site and seven genes in the surrounding area.

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