S100B Protein in Pro- and Antiapoptotic Doses Produces Different Effects on Defensive Behavior in Adult Rats

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We studied the effects of S100b protein in doses stimulating (500 and 50 ng) or inhibiting (5 ng) apoptosis in nerve cells on acquisition, retention, and retrieval of extinction of the acoustic startle response and conditioned fear in adult rats. After application to the vermis of the cerebellum S100b protein in doses of 500 and 50 ng impaired, while in a dose of 5 ng facilitated acquisition of both forms of defensive behavior. Different behavioral effects of S100b protein are probably related to its pro- and antiapoptotic effects on cerebellar cells relevant to the studied forms of behavior. Our results suggest that regulators of apoptosis are involved in the mechanisms of learning and memory.

Key Words: *apoptosis; behavior; memory; S100b protein*

In recent years studies of the neurochemical mechanisms underlying learning and memory were integrated with evaluation of the molecular basis for growth, development, and apoptosis (programmed cell death). Apoptosis plays an important role in the development of brain function. Dysregulation of apoptosis are responsible for various pathologies of the nervous system [14]. The same molecular factors are involved in integrative activity of the brain and proliferation, differentiation, and programmed death of cells [1,2,14]. One of these factors is neurospecific S100b protein, which possesses growth, trophic, and neuroprotective properties and provides specific functions of the brain [3,5,10,13]. However, S100b protein in high concentrations induces apoptosis in cultured nerve cells. Neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, and Down syndrome) are accompanied by accumulation of S100b protein in the nervous system, apoptosis in cells of certain brain structures, and cognitive dysfunction [8-11].

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Here we studied the effects of S100b protein in doses producing the pro- and antiapoptotic effect *in vitro* on acquisition, retention, and retrieval of various forms of defensive behavior in adult rats. S100b protein was applied to the cortex of the middle cerebellum relevant to these forms of behavior.

MATERIALS AND METHODS

Experiments were performed on adult Wistar rats weighing 250-300 g. The rats were kept in cages (3 animals per cage) and had free access to food and water. All measurements were conducted in the daytime (11.00-15.00).

Short-term and long-term extinction of the acoustic startle response (ASR) was studied by recording freezing behavior. Twenty-four hours before training the animals adapted to an experimental chamber for 5 min (no acoustic stimulation). On the next day they were maintained in the chamber for 5 min without acoustic stimulation. The freezing time was recorded. Twenty seconds later 10 strong sound signals were delivered against the background of wideband noise. After training the rats were returned to home cages. The animals were again placed in the chamber after

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24 h. The freezing time was recorded for 5 min. Long-term extinction was tested by delivering 10 sound signals with 20-sec intervals. ASR was studied in a special chamber connected to an automatic recorder and a computer via an electronic strain-sensitive amplifier. Freezing behavior was assessed visually. We estimated the duration of complete immobility (including nonmoving vibrissae) [5,6].

The acquisition and testing of defensive response in a given task were also evaluated in a brief study. Preadaptation was excluded. The period of training and testing in the chamber before stimulation was shortened from 5 to 3 min. The number of stimuli decreased from 10 to 3. Under these conditions ASR and conditioned fear did not extinguish in control rats. Therefore, it was possible to evaluate whether S100b protein in a dose producing the neurotrophic effect can potentiate learning and memory.

Two days before application of substances a hole was made above the cortex of the middle cerebellum and 2 mm caudal to the lambdoid suture (conditional line extending from the median suture) under ether anesthesia. S100b protein or physiological saline (3 μl) was applied to the cerebellar cortex in rats under light ether anesthesia. The solution was administered through the hole using a microsyringe with a needle stopper. We performed 3 series of experiments. In series I we evaluated the effects of S100b protein on learning and short-term memory in rats. S100b protein in doses of 500, 50, and 5 ng was administered 1 h before training. In series II S100b protein in the same doses was administered 2 h after training to determine its influence on consolidation/storage. In series III we determined the effect of S100b protein on retrieval. S100b protein in a dose of 500, 50, or 5 ng was administered 1 h before training. In a special series S100b protein in doses of 500 and 5 ng was administered 4 h before the training session. In this series the rats were repeatedly tested 72 h after the start of learning.

In control animals (10 rats per group) physiological saline (3 μl) was applied to the vermis of the cerebellum.

The data on ASR did not necessarily correspond to the normal distribution. Therefore, the results were analyzed by nonparametric Kruskal--Wallis ANOVA at $p<0.05$ (STATISTICA software).

RESULTS

Intact and control animals were characterized by short-term and long-term extinction of ASR. On day 2 the freezing time 2-fold surpassed that on day 1 (Tables 1 and 2).

S100b protein administered in a dose of 500 ng 1 h before training markedly decreased the amplitude of ASR (Table 1) and abolished short-term extinction. We revealed no differences in the amplitude of ASR to the first and tenth stimuli. S100b protein in a dose of 500 ng suppressed long-term extinction of ASR and conditioned fear. S100b protein in this dose had no effect on consolidation and retrieval of both forms of defensive behavior. A decrease in the amplitude of ASR was less pronounced after application of S100b protein in a dose of 50 ng 1 h before training. Other changes in learning and memory were similar after treatment with S100b protein in doses of 50 and 500 ng.

The decrease in the amplitude of ASR after application of S100b protein in high doses made the study of its effects during learning more difficult. A special series was conducted to avoid these changes. The training session started 4 h after administration of the preparation. Under these conditions the decrease

TABLE 1. Effect of S100b Protein Applied in a Dose of 500 ng to the Vermis of the Cerebellum on Acquisition, Retention, and Retrieval of Long-term Extinction of ASR and Conditioned Fear in Adult Rats ($M\pm m$, n=10)

Time of treatment, parameters	Intact		Control		S100b application	
	initial	after 24 h	initial	after 24 h	initial	after 24 h
1 h before training						
amplitude of first ASR	26.5±5.2	9.6±3.2*	25.3±2.6	11.3±3.3*	4.9±1.2+	3.1±0.9+
freezing time	41.9±5.7	66.8±4.9*	38.8±5.1	68.3±5.9*	53.2±5.5	45.4±4.4
2 h after training						
amplitude of first ASR	23.7±2.0	10.2±2.9*	24.3±2.0	12.4±2.5*	28.6±2.8	10.9±2.9*
freezing time	23.5±3.7	62.3±4.4*	28.7±3.7	67.3±5.1*	21.6±4.5	60.5±5.4*
1 h before testing						
amplitude of first ASR	24.9±3.8	12.5±4.1*	25.3±3.1	15.8±4.3*	23.4±3.5	14.3±3.5*
freezing time	29.5±3.2	72.4±2.1*	31.7±3.3	69.3±4.3*	31.8±3.6	67.5±5.1*

Note. Here and in Tables 2 and 3: $p<0.05$: *compared to initial value; +compared to the control.

in the amplitude of ASR produced by S100b protein in a dose of 500 ng was less pronounced (Table 2). However, S100b protein in a dose of 500 ng affected short-term extinction of ASR in both series. In rats receiving S100b protein 4 h before training the time of freezing and long-term extinction of ASR did not increase in the follow-up period (24 h later, Tables 1 and 2).

Changes in the initial amplitude of ASR became less significant with prolonging the interval between application of S100b protein in the apoptotic dose (500 ng) and start of training. However, under these conditions S100b protein had negative effects on learning and memory.

Retention of defensive response was tested 72 h after application of the preparation to evaluate the period of behavioral changes. In control animals the amplitude of the first ASR did not differ from that in test I (after 24 h), but was lower than the initial amplitude of ASR. The freezing time in control rats markedly surpassed the initial value. In animals treated with S100b protein in a dose of 500 ng the amplitude of the first response during repeated testing slightly surpassed the initial value, while freezing time did not increase. Therefore, changes in the behavior produced by S100b protein persisted for not less than 3 days (Table 2).

The initial amplitude of ASR in rats receiving S100b protein in a dose of 5 ng 4 h before training remained unchanged. However, short-term extinction of ASR in the day of training was impaired. No changes were revealed in long-term extinction of ASR 24 h after application of S100b protein (Table 2). The improvement was observed in finish learning after 72 h, which reflects facilitation of long-term memory. S100b protein in a dose of 5 ng had no effect on the initial characteristics and changes in freezing behavior.

Short training was not followed by acquisition of defensive behavior in control rats. Application of S100b protein in a dose of 5 ng 1 h before training promoted elicitation of the conditioned fear response, but did not modulate long-term extinction of ASR (Table 3). The protein had no effect on consolidation and retrieval. Application of S100b protein in a dose of 500 ng 1 h before training decreased the amplitude of ASR, but did not change other behavioral characteristics (similarly to normal training).

No differences were found between control and experimental rats receiving S100b protein 2 h after training. After application of S100b protein 1 h before testing the amplitude of ASR decreased compared to that observed on the day of training and under control conditions. Retrieval of the conditioned fear response remained unchanged (Table 3).

Our results indicate that S100b protein applied to the cerebellum in a dose inducing apoptosis in nerve cells impairs behavioral characteristics relevant to this brain structure (extinction of ASR and conditioned fear). However, application of S100b protein in a neurotrophic dose facilitates acquisition of conditioned fear produced by exogenous stimuli and promotes extinction of ASR during long training.

Neurospecific S100b protein in the proapoptotic dose suppressed defensive behavior, which is consistent with published data [4]. Our previous experiments showed that application of antibodies against various neurotrophic factors (CSL and R1 lectins and S100b and A3G7 proteins) and substances causing

TABLE 2. Effect of S100b Protein Applied in Doses of 500 and 5 ng to the Vermis of the Cerebellum 4 h before Training on Long-term Extinction of ASR and Conditioned Fear in Adult Rats ($M \pm m$, n=10)

Parameter, time of recording	500 ng			5 ng		
	intact	control	S100b	intact	control	S100b
Amplitude of first ASR						
initial	29.6±4.2	30.3±4.6	16.8±4.8^{+}	27.1±3.6	26.3±3.1	26.5±2.8
after 24 h	14.1±3.1*	15.8±3.3*	15.0±6.9	17.2±3.4*	14.6±3.5*	14.7±3.3*
after 72 h	14.9±2.3*	16.8±2.7*	23.2±5.5^{+}	16.9±2.6*	14.1±2.3*	12.8±3.1*
Amplitude of tenth ASR						
initial	18.3±2.1	19.6±1.9	18.2±2.3	16.2±1.9	15.6±2.3	23.7±2.6
after 24 h	12.2±1.1	11.3±1.0	10.7±0.9	10.3±1.2	10.8±1.3	9.7±1.1
after 72 h	9.1±0.8	8.3±0.9	20.5±1.5^{+}	8.9±0.9	9.6±0.7	6.4±0.5^{+}
Freezing time						
initial	38.2±6.4	34.3±5.0	40.5±7.8	48.1±7.2	44.6±4.9	41.6±6.7
after 24 ч	67.2±7.9*	71.4±8.5*	53.9±6.9	71.3±6.8*	69.4±6.3*	68.6±7.1*
after 72 h	85.0±4.2	91.7±6.7*	46.6±5.5^{+}	75.0±5.2	71.3±7.1*	69.4±6.5*

ischemic injury or changes in bioelectric activity of the nervous tissue (sodium azide and tetrodotoxin) to the vermis of the cerebellum decreases the amplitude of ASR and impairs acquisition of conditioned fear and short-term or long-term extinction of ASR [5, 6,15]. It should be emphasized that these bioactive substances stimulate apoptosis in brain cells [2,7]. Interestingly, transgenic animals with enhanced expression of S100b are characterized by disturbances in behavior and learning [12].

S100b protein in pico- and nanomolar concentrations possesses neurotrophic and neuroprotective properties and inhibits programmed death of nerve cells [10,13]. S100b protein facilitates acquisition of behavioral characteristics. Other neurotrophic factors also have neuroprotective activity and stimulate various forms of behavior [2].

It should be emphasized that behavioral changes produced by S100b coincided with programmed cell death in mature brain under the influence of apoptotic factors. The first signs of programmed cell death in the brain of adult animals were observed 1-3 h after apoptotic treatment and became most pronounced after 24-72 h [7].

S100b protein produces different changes in defensive behavior of adult rats, which is probably related to the pro- and antiapoptotic effect of this peptide in various doses on cells of the cerebellum. This brain structure is relevant to the studied forms of behavior. Our results indicate that endogenous regulators of apoptosis in brain cells (*e.g.*, neurospecific S100b protein) are involved in the neurochemical mechanisms of learning and memory.

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TABLE 3. Effect of S100b Protein Applied in Doses of 500 and 5 ng to the Vermis of the Cerebellum in Adult Rats on Acquisition, Retention, and Retrieval of Long-term Extinction of ASR and Conditioned Fear during Short Training ($M \pm m$, n=10)

Time of treatment, parameters		Intact		Control		S100b, 500 ng		S100b, 5 ng	
		initial	after 24 h	initial	after 24 h	initial	after 24 h	initial	after 24 h
1 h before training	amplitude of first ASR	37.8±5.2	31.8±4.3	35.3±5.6	35.2±6.3	6.2±2.9	5.8±2.5	37.8±7.2	38.2±7.9
	freezing time	36.5±5.9	38.8±4.9	37.8±5.1	38.1±5.7	43.2±5.5	51.1±6.8	49.3±4.9	64.4±5.4*
2 h after training	amplitude of first ASR	33.7±6.0	32.2±5.9	34.3±6.1	32.4±5.5	33.2±5.5	32.1±5.8	29.6±4.8	30.9±6.7
	freezing time	36.5±3.7	42.3±4.4	38.0±4.6	40.2±5.1	34.5±4.2	39.7±6.3	35.8±4.7	38.7±4.4
1 h before testing	amplitude of first ASR	35.9±6.8	32.5±4.1	29.3±5.1	35.8±4.3	33.2±5.5	11.4±5.8*	32.4±5.4	29.6±4.8
	freezing time	36.5±5.2	42.4±7.1	31.7±3.8	39.3±4.3	36.2±5.4	43.8±6.1	31.8±3.6	37.5±5.7

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