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Blood-brain barrier permeability differentiates Sadowski mouse lines of high and low stress-induced analgesia. Electron microscopy analysis

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Abstract

The blood-brain barrier (BBB) forms a filtering system between peripheral circulating blood and the central nervous system. Pathological leakage of the BBB is probably responsible for various dysfunctions and diseases. Over twenty years ago, Sadowski et al. separated two lines of mouse, one with high sensitivity (HA) and the other with low sensitivity (LA) to stress-induced analgesia (SIA). We propose that leakage of the BBB is responsible for the difference in SIA of the "Sadowski mouse" model. The presented BBB electron microscopy structural analysis of both lines provided evidence for this hypothesis. Up to now, a good natural animal model of differences of BBB permeability is not known. The "Sadowski mouse" may fulfil this deficiency.

Key words: *blood-brain barrier, Sadowski mouse, stress-induced analgesia, ultrastructure.*

Introduction

The blood-brain barrier (BBB) is often used as a general expression for biological barriers between the central nervous system (CNS) and the periphery that select nutrients necessary for CNS function, eliminate metabolic products, and protect the central nervous system from pathogens circulating in the periphery, bacterial and viral infections, as well as separating the CNS from peripheral immunological components. Although the important role of BBB in chemical signalling in interaction of the periphery with the CNS in behaviours like food intake or aggression is already recognized and is under extensive study, the knowledge on the BBB in cross-interaction between the CNS and periphery is only fragmental.

Stress-induced analgesia is the suppression of pain sensitivity upon exposure to a stressful stimulus. This behavioural phenomenon has been known for over 30 years [5] and is extensively studied. Pharmacological and neurochemical studies have concluded that most of all known neurotransmitters take part in modulation of pain perception [4]. CNS-

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mediated stress-induced analgesia often is a result of stressful changes in the periphery. Therefore, the strong role of BBB status in generation of stress-induced analgesia and its individualization could be expected.

Over twenty years ago, Sadowski *et al.* separated two lines of mouse, one with high sensitivity (HA) and the other with low (LA) expression of stress-induced analgesia (SIA) initiated by swim stress, followed by measurement of analgesia with the hot-plate and tail-flick tests [13,14]. Recently, with conviction of the importance and usefulness of this "mouse model" in further pharmacological and physiological studies, we started naming both lines the "Sadowski mouse". During the following years both lines were extensively screened for biochemical differences. Although these studies identified some phenotypic differences [11,12,15-20], none of them fully explained the differences in stress-induced analgesia.

An increased level of circulating peripheral endogenous peptides, including endorphins, is known to play a major role in stress-related behaviours [2,9]. To induce analgesia, circulating peptides have to reach CNS structures. We developed the hypothesis that differences in leakage of the blood-brain barrier in the Sadowski mouse could be the major difference between selected LA and HA lines. We hypothesized that HA mice have much more leakage of the BBB than the LA line. As a consequence, blood circulating endogenous opioid peptides increased by stress could more easily penetrate the CNS in the HA line and induce central analgesia. A tight BBB in the LA line prevents peripheral endogenous opioid peptides permeation into the CNS and, in consequence, prevents stress-induced analgesia. To obtain evidence for our hypothesis we analysed the structure of the blood brain barrier by electron microscopy.

Material and methods

The animals were handled according to the guidelines of the local ethics committee for experimentation on animals.

Blood-brain barrier permeability

Stress-induced analgesia (SIA) of Swiss-Webster HA and LA mice lines was evaluated by the method published previously [18] using post-swim hot-plate assay. A detailed description of the HA and LA lines has been presented elsewhere [13]. Briefly, outbred mice were exposed to 3-min swimming in 20°C water, and 2 min after completion of the swim were measured for the latency of a nociceptive reflex on a hot plate at 56°C. Males and females displaying the longest (50-60 s) and the shortest (< 10 s) post-swim latencies of the hind paw flick or lick response were chosen as progenitors of the HA and the LA line, respectively. A similar procedure was repeated in each offspring generation, and only subjects displaying the longest and the shortest post-swim hot-plate latencies were mated to maintain, respectively, the HA and the LA line. Unselected Swiss-Webster mice were taken as the control group.

Ultrastructural analysis

Mice were anaesthetized and perfused transcardially with saline followed by 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer pH 7.4. Tissue sections from the cerebral cortex were additionally fixed in the same fixative for 2 hours at 4°C. After washing, the slices were post-fixed in 1% OsO_4 for 30 min. Afterwards, the slices were dehydrated in graded ethanols, and embedded in Epon 812 resin. For electron microscopy, ultrathin sections were processed by cutting with a diamond knife on a Reichert Ultramicrotome and then collected on copper grids. The material was air-dried, then stained for 10 min with 4.7% uranyl acetate and for 2 min with lead citrate. The sections were examined and photographed in a JEOL 1011 electron microscope (JEOL Ltd. Tokyo, Japan).

Results

Control mice

Our morphological, electron microscopy studies were focused on all structural and cellular elements of the blood-brain barrier (BBB) composed of endothelial cells, with a tight junction, close, solid basement membrane, pericytes and astrocytes or their processes around capillaries. All structural elements of the BBB were packed very tightly between them (Fig. 1). Structural elements of neighbouring neuropil have numerous, countless processes of both nerves (dendrite, axon) and glial (protoplasmic, fibrillar astrocyte and oligodendrocyte) cells, with numerous synapses and strong connections between them. Ultrastructural analysis revealed no abnormal-

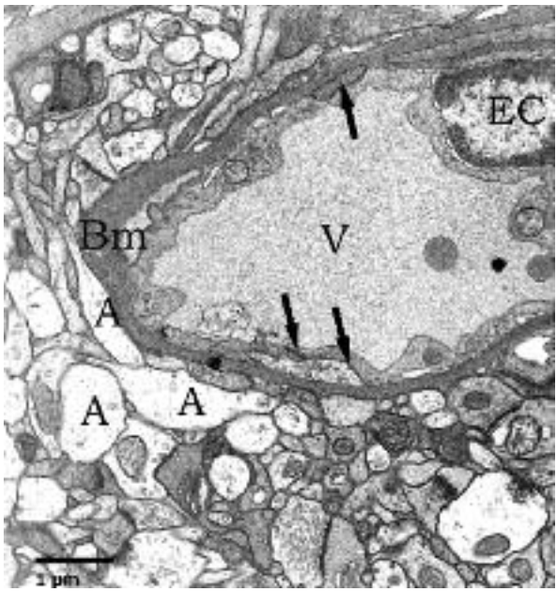

Fig. 1. Control mice – cerebral cortex. Part of brain with detail of blood-brain barrier (BBB); the thin endothelial cells (EC) with tight junction (arrows) between them, very solid basement membrane (Bm) and astrocytes or their processes (A) closed apposed to the Bm-forming perivascular layer. All elements of BBB are ultrastructurally unchanged. The vessel (V) is densely covered by various structured elements of neuropil. Note close integration of all structural elements of the brain. Bar = 1 µm.

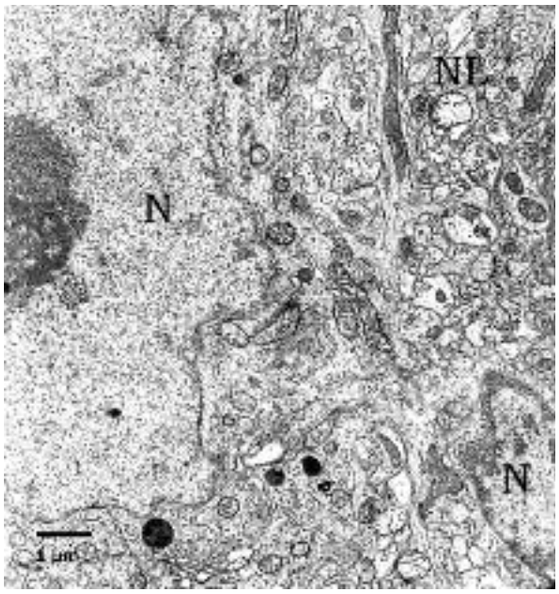

Fig. 2. Control mice – cerebral cortex. Cerebral cortex. Fragment of nerve cells (N) ultrastructurally normal. In neuropil (NL) close connections between all structures without any ultrastructural abnormalities are present. Bar = 1 µm.

ities in any structural or cellular elements of the BBB or of the brain's neuropil. Close connection between all cellular structures and structural elements of neuropil is evidenced (Figs. 1-3).

HA mice

Histological studies in HA mice revealed many reliable ultrastructural abnormalities for structural and cellular elements of BBB and neuropil.

Most frequently, disruption of the BBB was expressed. Characteristic massive, large spaces (asterisk) between capillaries and neuropil were observed. In such capillaries, endothelial cells and basement membrane were extremely thin. The thinnest parts of endothelium (such as capillary pores), and present merely fenestrations (arrows), each of which is bridged by a thin diaphragm, and lack of a tight junction between endothelial cells, indicate their

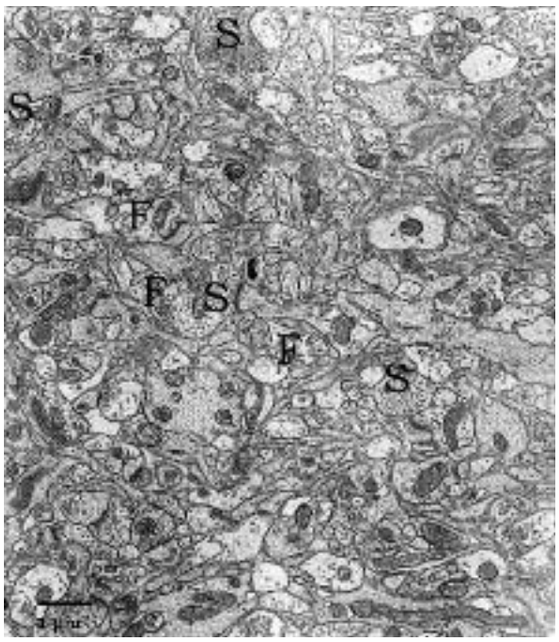

Fig. 3. Control mice – cerebral cortex. In neuropil tight connections between all structural elements and two types of synapses, with flattened vesicles (F-type) or round vesicles (S-type), could be seen. Bar = 1 µm.

discontinuities and disturbances in exchange of substances across the cell (Fig. 4).

Many capillaries presented a very irregular shape, multiplication of basement membrane, or partial loss of their trilaminar construction, with lack of a tight junction between endothelial cells and morphological characteristics of apoptosis for endothelial or pericyte cells. Massively swollen astrocytes or astrocytic processes with scarce organelles and glycogen particles and narrow capillary lumen were seen (Figs. 5-7).

The striking and reliable ultrastructural abnormalities in neuropil of the brain HA mice line were represented by swollen synapses or dendrites. Two types of synapses (S-type with round synaptic vesicles and F-type with flattened vesicles) were distinguished. Several synapses in the final phase of degeneration (drastic reduction of synaptic vesicles or their aggregation, swollen synaptoplasm, change in their shape or size) were seen in the neuropil surrounding capillaries. Between them ultrastructurally changed neurons or so-called "dark neurons" occurred sporadically (Fig. 8).

LA mice

Generally, in this experimental group of animals, all cellular elements of the BBB were ultrastructurally unchanged. We noted total lack of fenestrations in endothelium but frequently tight junctions linking endothelial cells between them were seen. Sporadically, shrinkage of endothelial cells and slightly swollen synapses or astroglial cells and their processes were seen. Between such capillaries the microglial cells were seen. We also observed capillaries with morphological alteration characteristic for angiogenesis, as another new event. Young capillaries with hypertrophic endothelial cells with numerous tight junctions, solid basement membrane and very narrow lumen were frequently present (Fig. 9).

"Dark neurons" were never seen and structural elements of neuropil, like synapses or dendrites, and neuronal cells were well preserved (Fig. 10).

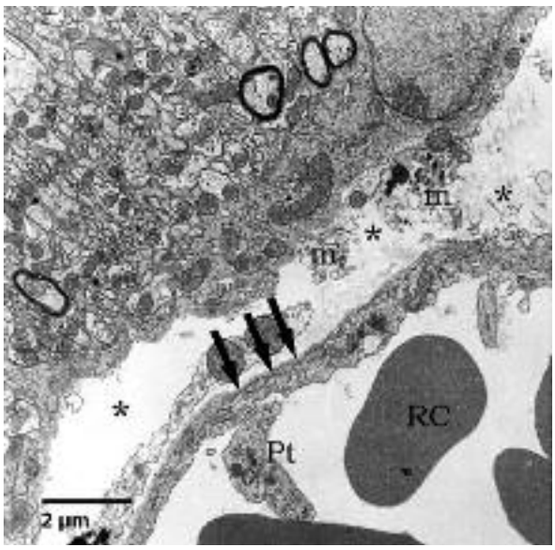

Fig. 4. HA mice – cerebral cortex. Note extremely thin endothelium with fenestration (arrows) but without any tight junction in the close proximity between platelets (PT) and red cell (RC). In large, electron-empty space (asterisk), remnant of cellular material (m) of endothelial degenerating cells is present. All elements of neighbouring neuropil are ultrastructurally unchanged and have close contacts between them. Bar = 2 µm.

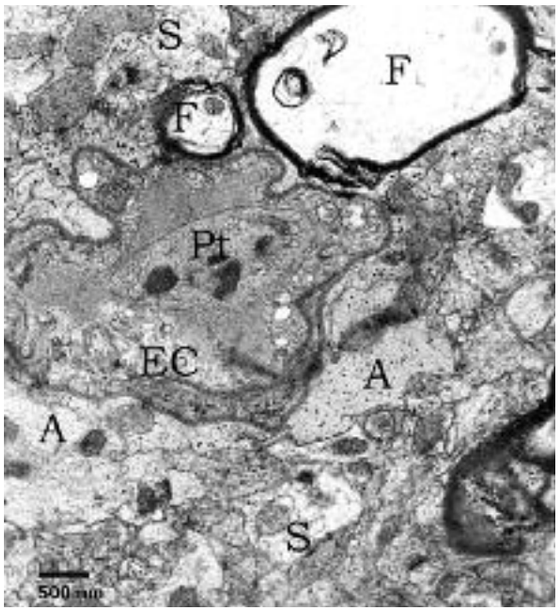

Fig. 5. HA mice – cerebral cortex. Note capillary with very irregular shape and abnormal ultrastructure of endothelial cell (EC) with lack of tight junction. A platelet (Pt) in lumen of capillary is seen. Multiplication and invagination of basement membrane and swelling neighbouring astrocytes (A) or synapses (S) or myelinated nerve fibres (F) in neuropil are seen. Bar = 500 nm.

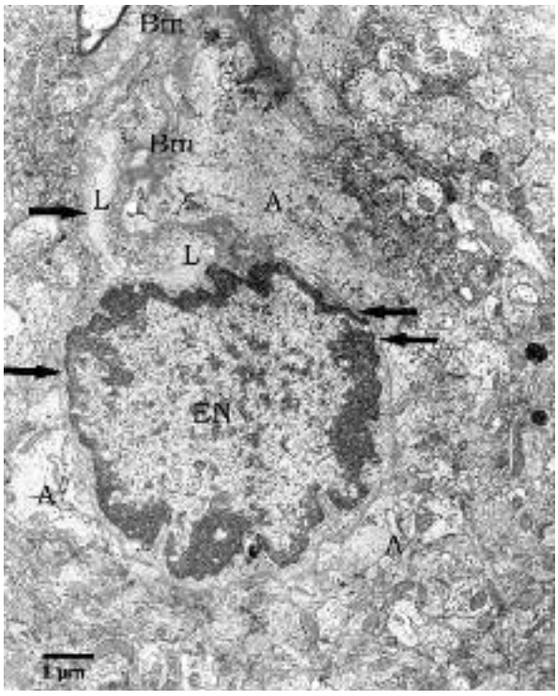

Fig. 6. HA mice – cerebral cortex. Irregular capillary with enormous nucleus and thin cytoplasm of endothelial cell (arrows) and narrowed lumen (L) caused by swollen astrocytes (A) and multiplication of basement membrane (Bm) with loss of their trilaminar construction are seen. Bar = 1 µm.

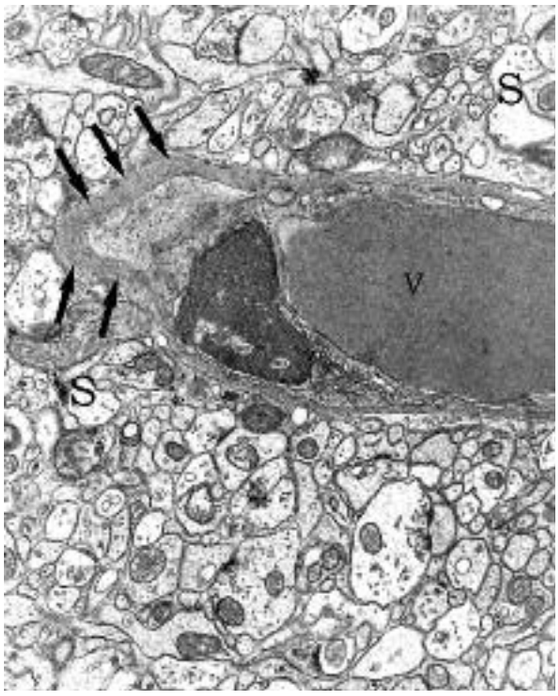

Fig. 7. HA mice – cerebral cortex. Endothelial cells of capillaries (V) have characteristic apoptotic nucleus (N) and basement membrane shows frequently broken down trilaminar construction (arrows). Degeneration of some synapses (S) is seen also. Bar = 1 µm.

We suggest that a well-preserved BBB and dense new vessel formation may facilitate protection against ultrastructural alterations of brain in LA mice, compared to brain of HA mice with a leaking blood-brain barrier.

Our morphological, electron microscopy observation also showed that many nerve terminals (synapses) and dendrites are the prime target for ultrastructural alteration or degeneration without necessarily killing the entire nerve cell or some other elements of the neuropil. We suppose that this effect could be the result of genetic dysfunction or impairment of BBB development in the newborn mouse.

Discussion

The blood-brain barrier (BBB) is a very protective structure that protects the brain from exposure to both endogenous and exogenous compounds entering the brain parenchyma from the circulation [1]. Pathological leakage of the BBB could be the primary cause of several pathologies and diseases such as multiple sclerosis [7], Alzheimer and Parkinson diseases [8,22], eating disorders [10] or in congenital hydrocephalus [6]. The BBB is created by tight junctions between the cerebral endothelial cells, the choroid plexus epithelial cells and the cells of the arachnoid epithelium. Extremely tight 'tight junctions' are a key feature of the BBB, which significantly reduce permeation of polar solutes through paracellular diffusional pathways between the endothelial cells from the blood plasma to the brain extracellular fluid [3,21].

We suggest that a well-preserved BBB and dense new vessel formation may facilitate protection against ultrastructural alterations of brain in LA mice, compared to brain of HA mice with a leaking blood-brain barrier. In contrast to LA and control mice, in HA mice the disruption of the BBB was significantly expressed. In mice from the HA line, chara-

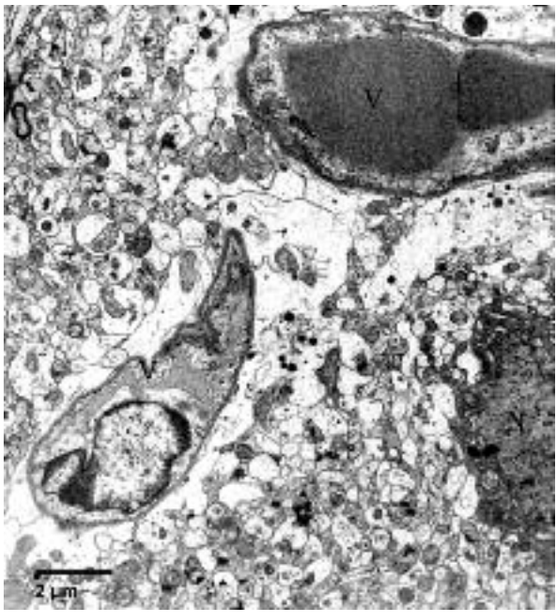

Fig. 8. HA mice – cerebral cortex. In this fragment of cerebral cortex characteristic morphological features are visible: degeneration of vessels (V), neuron (N) and many elements of neuropil (synapses, dendrites, astrocytes). Bar = 2 µm.

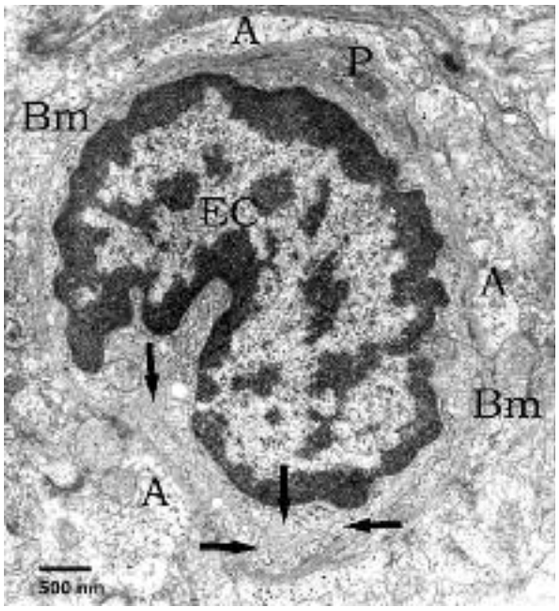

Fig. 9. LA mice – cerebral cortex. New vessel formation with characteristic features for angiogenesis; hypertrophic endothelial cell (EC) connected between them by tight junction (asterisk) with very narrow or no open yet lumen (arrow), solid basement membrane around capillary and pericyte (P) and astrocyte cells closely apposed to the basement membrane are present, suggesting their protection against ultrastructural alterations. Bar = 500 nm.

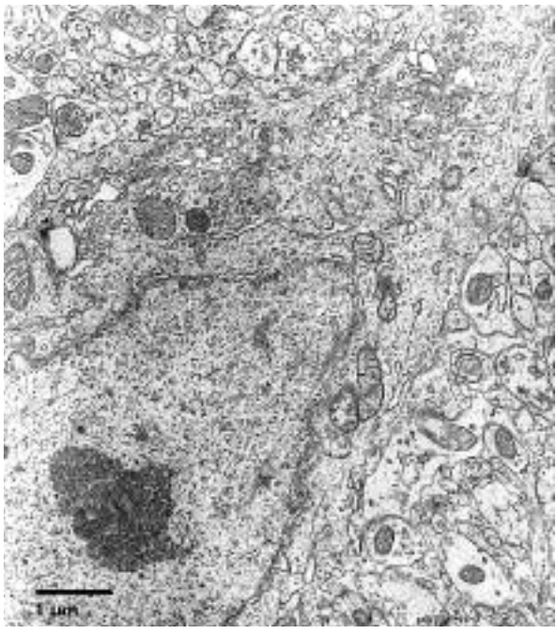

Fig. 10. LA mice – cerebral cortex. Note all cellular and structural elements of the brain very well preserved and ultrastructurally unchanged. Bar = 1 µm.

cteristic massive, large spaces, (asterisk) between capillaries and neuropil were observed. In such capillaries, endothelial cells and basement membrane were extremely thin. The thinnest parts of endothelium (such as capillary pores), and present merely fenestrations, each of which is bridged by a thin diaphragm, and the lack of a tight junction between endothelial cells, indicate their discontinuities and disturbances.

Our electron microscopy morphological observation also showed that many HA nerve terminals (synapses) and dendrites are the primary target for ultrastructural alteration or degeneration without necessarily killing the entire nerve cell or some other elements of the neuropil. It should be taken into account that these changes could also participate in stress-induced analgesia.

Therefore, it could be concluded that BBB pathophysiological conditions of HA and LA Sadowski

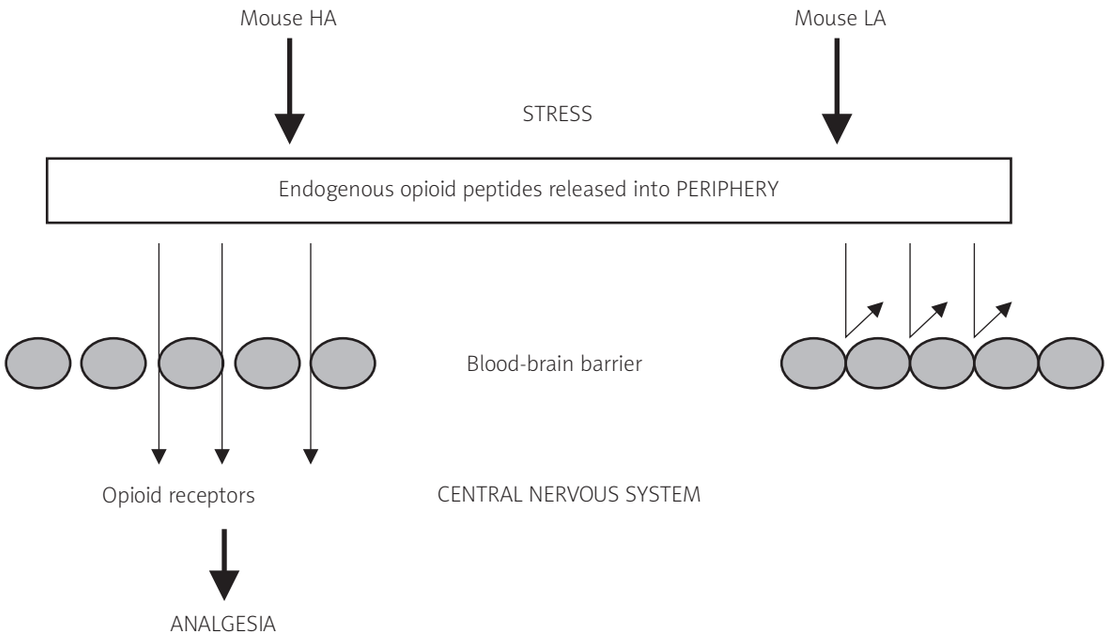

Fig. 11. Schematic summarization of stress effects on HA and LA mouse lines. Stress induces increase of endogenous opioid peptide level in periphery. These peptides are able to penetrate licking (HA) BBB and activate opioid receptors in CNS. Well-developed BBB (LA and Control) are tight, impermeable for peptides.

mouse lines are significantly different. The structure of the LA line is related to the healthy, control group of Swiss-Webster mice, whereas the HA line is characterized by BBB leaking components. Although additional experiments are in progress, the BBB ultrastructural differences between HA and LA mice indicate that BBB leakage is a cause of high response to stress-induced analgesia (SIA) of the HA line. Stress elevates peripheral endogenous opioid peptides (e.g. beta-endorphins). The SIA phenomenon is observed as a result of central activity of endogenous opioid peptides influx from the periphery (Fig. 11).

However, the condition of the BBB is not limited to the opioid system. Therefore the two lines of "Sadowski mouse" could be used as a good general model in studies of natural differences in BBB permeabilities of various components, such as new drugs, viruses, antibodies, etc. Up to now, a good natural animal model of differences of BBB permeability is not known. We believe that the "Sadowski mouse" fulfils this deficiency.

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