Research report

Hyperexcitability of amygdala neurons of Senescence-Accelerated Mouse revealed by electrical and optical recordings in an in vitro slice preparation

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Abstract

In the amygdala (AMG) slices obtained from both the young (4–7 months old) and aged (17–20 months old) groups of Senescence-Accelerated Mouse (SAM) P10, spontaneous bursts were recorded in the medial, central and basolateral nuclei. The spontaneous bursts were also observed in the slices from the young group of SAMR1, whereas the mean frequency was significantly lower than that from the young group of SAMP10. The spontaneous burst was barely detectable in slices from the aged group of SAMR1 during perfusing with the standard solution, while bicuculline methiodide 10 μM, a GABA A receptor antagonist, or Mg 2+-free solution induced a similar bursting activity observed in the young group. The burst response was also evoked in the medial, central and basolateral AMG following stimulation of the stria terminalis (ST). Both spontaneous and evoked bursts were completely suppressed by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 4 μM), an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor antagonist, but not by (+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine hydrogen maleate (MK-801, 30 μM), an N-methyl-D-aspartate receptor antagonist. The hyperexcitability of the AMG neurons was further substantiated by optical recordings. Following stimulation of the ST, the optical signals reflected postsynaptic responses spread into the medial and central AMG areas at 2–5 ms and faded out at 20–30 ms after stimulation. The intensity of the optical signal recorded in the slice from the young SAMP10 was significantly higher than that from SAMR1 or ddY mice. These observations indicate that bursts mediated by AMPA/kainate receptors were transiently generated in the AMG of SAMR1 at the young age, while the bursts with higher frequency were continuously generated in the AMG of SAMP10. The chronic neuronal hyperactivity in the AMG may be partially responsible for the age-related deterioration of memory and learning abilities observed in SAMP10. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Senescence-Accelerated Mouse; Amygdala; Electrophysiology; Optical recording; Slice preparation

1. Introduction

Senescence-Accelerated Mouse (SAM) consisted of two strains has been established as a murine model of accelerated senescence [25]. One strain (SAMP) is prone to accelerated senescence with a shortened life span, while the other strain (SAMR) is resistant. In the SAMP series, SAMP10 has been shown to exhibit pathological changes in the brain such as atrophy particularly predominant in the forebrain and the olfactory bulb [20,21] and spongiform degeneration in the brainstem [1]. Furthermore, SAMP10 has been reported to show an age-related impairment of learning and memory [21,22]. In particular, the aged SAMP10 failed in the avoidance behavior freeze instead of running to avoid the shock, while young SAMP10 and all ages of SAMR1 can avoid the shock by learning to block the freezing [20].

The amygdala (AMG) is a subcortical formation of the limbic system and has been implicated to play a crucial role in certain types of learning and memory besides a variety of emotional and motivational aspects of behavior [2,18]. The AMG receives excitatory inputs which are mediated by both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate and N-methyl-D-aspartate (NMDA) receptors [2,11,14,16]. There is increasing evidence that both AMPA/kainate and NMDA receptors
in the AMG are involved in the conditioned fear [2,8,9]. Therefore, it is interesting to speculate that the inhibitory modulation of fear responses observed in the aged SAMP10 results from dysfunction of the glutamatergic transmission in AMG neurons. In the present study, we attempted to elucidate possible alterations in the glutamatergic transmission of the AMG neurons in SAMP10 and SAMR1 by a combination of electrical and optical recordings in the in vitro slice preparations.

2. Materials and methods

2.1. Animals

Two age groups (young: 4–7 months; aged: 17–20 months) of SAMP10 (SAMP10//Sea) and SAMR1 (SAMR1TA//Sea) and 4-month old ddY mice were obtained from Seac Yoshitomi, Fukuoka, Japan and bred under conventional conditions at temperature of 24 ± 1°C with 12-h light–dark cycle. They were housed in groups in cages and allowed free access to food and water. SAMR1 was used in the present study as a control senescence-resistant inbred strain. In some experiments, ddY mice were also used as a control.

2.2. Preparation of slices

This study was approved by the Animal Research Committee of the Kyushu University Faculty of Dentistry and Faculty of Pharmaceutical Sciences. Animals were decapitated under light ether anesthesia and brains were rapidly removed and placed in ice-cold Krebs–Ringer solution of the following composition (mM): NaCl 124.0, KCl 5.0, KH₂PO₄ 1.24, NaHCO₃ 26.0, CaCl₂ 2.4, MgSO₄ 1.3, and glucose 10.0. Each brain was trimmed with a razor blade to a block containing the region of the AMG complex. Coronal slices (400 µm thick) containing the AMG and the stria terminalis (ST) were cut from the block with a vibroslice (Vibroslice 752 M, Campden Instruments, Cambridge, UK). AMG slices were preincubated in an interphase-type reservoir perfused with Krebs–Ringer solution equilibrated with 95% O₂ and 5% CO₂ gas mixture for at least 1 h.

2.3. Electrophysiological study

Nine young and 11 aged SAMP10, 11 young and 11 aged SAMR1 and nine ddY mice were used for electrophysiological studies. The detailed methods for recordings

Fig. 1. Spontaneous bursts recorded in medial AMG neurons from the young SAMP10. (A, B) Traces in (A) and (B) are simultaneous extracellular and intracellular recordings, respectively. (C) Spontaneous bursts recorded during injections of continuous depolarizing and hyperpolarizing currents. Current intensity is indicated on the right of each of the traces.
Fig. 2. The mean frequency of spontaneous burst recorded from the medial AMG of SAMP10, SAMR1 and ddY mice. Each column and bar represents the mean and S.E.M., respectively. The number of animals examined is shown in parenthesis. *$P<0.05$, ***$P<0.0005$ as compared with the young group of the same strain (Student’s $t$-test). 

Fig. 3. Effects of MK-801 and CNQX on the evoked (A) and spontaneous bursts (B) recorded from the medial AMG of the young SAMP10. Voltage calibration in (A) also apply to (B). An arrowhead indicates the onset of the ST stimulation.

have been described elsewhere [11,23]. Briefly, the AMG slice was placed in an interphase-type recording chamber at a constant bath temperature of 36°C. The position of the subnucleus of the AMG could be identified in the slice under the microscope by the location of the ST and the optic tract. Electrical stimulation (intensity 5–15 V, 200 μs duration, 0.1 Hz) was applied through a bipolar stainless steel electrode gently placed on the ST, a major afferent and efferent AMG pathway. Extracellular field potentials were recorded with a glass electrode filled with the perfusate and placed on the surface of the slice. The average of four consecutive response was displayed on a digital storage oscilloscope and plotted on an $X$–$Y$ plotter. Intracellular recordings from AMG neurons were obtained through a glass microelectrode filled with 2 M K-acetate and a high impedance amplifier (Neurodata IR 183). Electrical responses were stored in a videocassette recorder through a PCM data processor (VR-10B, Instrutech) and plotted on an $X$–$Y$ plotter. (+)-5-Methyl-10,11-dihydro-5H-dibenzo-[$a,d$]-cyclohepten-5,10-imine-hydrogen maleate (MK-801, Research Biochemicals International, Natic, MA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris Cookson, Bristol, UK), bicuculline methiodide (Sigma, St. Louis, MO) and tetrodotoxin (TTX, Sankyo,
Tokyo, Japan) were added to the perfusing medium. Mg\(^{2+}\)-free Krebs solution was made by eliminating MgSO\(_4\) without replacement.

2.4. Optical recordings

Four young SAMP10, three young SAMR1 and four ddY mice were used for optical recordings. After preincubation, AMG slices were stained with RH-482 (Nippon Kanko-Shikiso Kenkyusho, Okayama, Japan) at a concentration of 0.02% for 30 min. The stained AMG slice was transferred to a submersion-type recording chamber on an inverted microscope (IX70 Olympus, Tokyo, Japan) and was held at the bottom of the chamber by two small, thin silver weights. The temperature of the chamber was kept constant at 30°C by means of an electrically regulated heating system. The chamber was continuously perfused with Krebs solution at a rate of 1–2 ml/min. Electrical stimulation (intensity 5–15 V, 200 μs duration) was applied through a bipolar stainless steel electrode gently placed on the ST. The responses in the AMG evoked by electrical stimulation of ST were recorded as a change in light absorption of the voltage-sensitive dye RH-482 by an optical recording system (HR Deltaron 1700, Fuji Photo Film, Tokyo, Japan) consisted of a 128 × 128 photodiode array and a processing unit [5,6]. An interference filter of 700 ± 30 nm (BPF-4, Vacuum Optics, Japan) was placed in the light path using a tungsten–halogen lamp (150 W) as the light source because RH-482 showed the peak absorption at about 700 nm. The duration of light exposure was controlled by an electromagnetic shutter (Chuo-seiki, Tokyo, Japan) to avoid photodynamic damage and dye bleaching. With the 4 × objective used in this study, each photodiode corresponded to the tissue area of 17.5 × 17.5 μm, and the whole array corresponded to that of 2.24 × 2.24 mm [7]. The microscope and camera assembly were placed on an air suspension table to isolate them from external vibrations. Before stimulation, a 128 frame average image of background light signal was stored in a reference memory. The stored reference data were continuously subtracted from real-time images and transferred to the unit sequentially with a temporal resolution of 0.6 ms/frame. One trial consisted of 512 real-time images, and 16 trial images were averaged to improve the signal-to-noise ratio.

![Fig. 4. Effects of bicuculline methiodide and Mg\(^{2+}\)-free solution on evoked (A) and spontaneous activities (B) recorded from the medial AMG of the aged SAMR1. Voltage calibration in (A) also apply to (B). An arrowhead indicates the onset of the ST stimulation.](image-url)
3. Results

3.1. Age-related changes in spontaneous burst activity in the AMG

In the AMG slices from the young group (4–7 months) of SAMP10, spontaneous bursts were recorded in the medial, central and basolateral nuclei of the AMG. Fig. 1 shows the typical example of spontaneous burst recorded in the medial AMG from the young SAMP10. The spontaneous burst recorded extracellularly consisted of a large negative potential (1–3 mV) followed by a positive potential with small amplitude (0.2–0.5 mV) (Fig. 1A). Simultaneous intracellular recordings revealed that the negative and positive waves corresponded to the depolarization shift and the after-hyperpolarizing potential, respectively (Fig. 1B). The spontaneous firing was markedly increased during application of continuous depolarizing currents, whereas the frequency of the spontaneous burst was not significantly affected by the membrane hyperpolarization (Fig. 1C). In the young group of SAMR1, spontaneous bursts were also recorded in the AMG, while the mean frequency of spontaneous burst recorded in the medial AMG was significantly lower than that from the young group of SAMP10 (Fig. 2). To assess age-related changes in the frequency of spontaneous bursts, extracellular recordings were also made in the medial AMG from the aged group (17–20 months) of SAMP10 and SAMR1. In the aged group of SAMP10, the mean frequency was significantly lower than that recorded from the young group of SAMP10. On the other hand, there was no observable spontaneous burst in the medial AMG from the aged group of SAMR1. In ddY mice, no burst response was recorded. Similar results were obtained when the mean frequency of spontaneous bursts recorded from the central or basolateral AMG was compared among SAMP10 and SAMR1 with different two age groups (data not shown).

Among the subnucleus of the AMG, electrical stimulation of the ST evoked burst responses with the largest amplitude in the medial nucleus. The central nucleus of the AMG also exhibited the burst responses, while it was difficult to evoke stable bursts in the basolateral nucleus. The evoked responses consisted of a large negative potential (2–3 mV) followed by oscillatory negative potentials with a small amplitude. Intracellular recordings also re-

Fig. 5. A pattern of optical signal propagation observed in the AMG of the young SAMP10 evoked by electrical stimulation of the ST. The schematic drawing in the upper left corner shows the relative positions of the preparation shown by a real camera image. Numerals in each image correspond to time before and after the onset of stimulation. The fractional absorbance change was monochrome-coded, as shown by the pseudo-monochrome scale in the right. ST, stria terminalis; OT, optic tract; CE, central amygdala; ME, medial amygdala; BL, basolateral amygdala.
revealed that the large negative waves corresponded to the depolarization shift (data not shown). Since the involvement of glutamate receptors in burst generation in the AMG has been well demonstrated [3,4,11,17], effects of glutamate receptor antagonists on evoked and spontaneous bursts in the medial AMG from the young group of SAMP10 were examined. Bath application of MK-801 (30 μM), a noncompetitive NMDA receptor antagonist, was ineffective in blocking the evoked burst or reducing the frequency of spontaneous burst (Fig. 3) \((n = 5)\). By contrast, CNQX (4 μM), a competitive AMPA/kainate receptor antagonist, completely suppressed both evoked and spontaneous bursts (Fig. 3) \((n = 5)\). During application of CNQX, electrical stimulation of the ST still evoked a fast-spike like potential followed by a negative potential with small amplitude. These responses may reflect the spike activity of axons at the recording foci or the antidromic activity of medial AMG neurons since bath application of TTX (1 μM) completely eliminated them (data not shown).

In the AMG slices from the aged group of SAMR1, electrical stimulation of the ST evoked a fast spike-like potential followed by a negative potential with a small amplitude and no spontaneous burst was observed in the medial AMG. When bicuculline methiodide was applied to the perfusing solution with a final concentration of 10 μM, both evoked and spontaneous bursts were induced (Fig. 4) \((n = 4)\). Mg\(^{2+}\)-free solution also induced both evoked and spontaneous bursts \((n = 4)\). The evoked or spontaneous bursts induced in these conditions were completely blocked by CNQX (4 μM) but not by MK-801 (30 μM) (data not shown).

### 3.2. Optical responses induced by the ST in the AMG

Following electrical stimulation of the ST, the optical signals first propagated on the medial nucleus and then propagated on the central nucleus in the AMG slices from the young P10, while only weak or no optical signal propagated on the basolateral nucleus. The optical responses until 2 ms after stimulation was considered to be due largely to a direct activation of neurons and axons since the peak latency of CNQX-sensitive postsynaptic responses was 2–5 ms. The following spreading responses were considered to be derived from the postsynaptic responses and faded out at 20–30 ms after stimulation. The

Fig. 6. A pattern of optical signal propagation observed in the AMG of ddY mouse evoked by electrical stimulation of the ST. The schematic drawing in the upper left corner shows the relative positions of the preparation shown by a real camera image. Numerals in each image correspond to time before and after the onset of stimulation. The fractional absorbance change was monochrome-coded, as shown by the pseudo-monochrome scale in the right. ST, stria terminalis; OT, optic tract; CE, central amygdala; ME, medial amygdala; BL, basolateral amygdala.
intensity of optical responses recorded in the AMG slices from ddY mice was relatively weak as compared with those from the young SAMP10. The relative averaged light absorption change at 2.4 ms after stimulation in the AMG of the young SAMP10 and SAMR1 as compared with that of ddY mice was 305.01 ± 64.9 (mean ± S.D., n = 4) and 113.0 ± 14.8% mean ± S.D., (n = 3), respectively (Figs. 5 and 6).

4. Discussion

The major finding of the present study is that the medial and central AMG neurons of SAMP10 as well as SAMR1 showed both evoked and spontaneous bursts which were never observed in the same region of ddY mice. The frequency of the spontaneous burst of SAMP10 was significantly higher than that of SAMR1. The frequency of spontaneous bursts recorded in the AMG of SAMP10 was significantly decreased with aging and no spontaneous bursts were observed in the aged group of SAMR1. To confirm the neuronal hyperexcitability detected in AMG neurons of SAMP10 by electrical recordings, the synaptic activity was also analyzed with optical recordings. The optical recordings were conducted in the AMG slices stained with the voltage-sensitive dye, RH482, which binds to excitable membranes and acts as a molecular transducer that transforms fast changes in membrane potential into optical signals. Electrical stimulation of the ST induced optical responses which propagated to the medial and then the central nucleus of the AMG. The relative averaged light absorption change in the AMG of the young SAMP10 was significantly larger than that of the young SAMR1 or ddY mice. These observations obtained from combination of electrical and optical recordings strongly suggest that the AMG neurons of SAMR1 and SAMP10 are hyperexcitable states especially at their early age. Although the signs of spontaneous convolution were not observed in either SAMP10 or SAMR1 in the present study, our preliminary experiments showed that the young SAMP10 and SAMR1 had a lower threshold for kainate-seizure as compared with ddY mice.

The hypothalamus is the most possible origin of the excitatory post-synaptic potential (EPSP) recorded in the AMG slices since the hypothalamic projections to the AMG through the ST are known to terminate mainly within the medial and central nuclei [15,19]. In the medial AMG, we have previously shown that local stimulation evoked the dual component of EPSP consisted of both a fast AMPA/kainate- and a slow NMDA-receptor mediated component [11]. In the AMG from SAMP10 and SAMR1, the AMPA/kainate receptors are considered to be of prime importance and NMDA receptors are not required for the burst activity since both the spontaneous and evoked bursts were suppressed by CNQX but not by MK-801. These our observations are consistent with the findings that epileptiform bursts elicited by kainate in the CA3 hippocampus region could be blocked by CNQX [12] but not by NMDA receptor antagonists [13]. However, it is also known that NMDA antagonists could not suppress even the NMDA-receptor dependent burst activity once it is established [24]. Our observations showed that bicuculline-containing or Mg²⁺-free Krebs–Ringer solution induced similar evoked and spontaneous bursts in the medial AMG of the aged SAMR1. These manipulations are known to make conditions favorable for activation of NMDA receptors, whereas NMDA receptor antagonists failed to block either evoked or spontaneous bursts. Therefore, the involvement of NMDA receptors in the bursts generation in the AMG of the SAMP10 and SAMR1 cannot be totally ruled out.

We have previously reported that burst discharges were evoked by a local stimulation in the medial AMG of the olfactory bulbectomized rat [11]. The reduction of GABAergic inhibition was considered to be mainly responsible for the hyperexcitability of medial AMG neurons since the IPSP amplitude and GABA content in the medial AMG was significantly decreased following the olfactory bulbectomy in the rat. Furthermore, we have also observed that the sub-spike stimulation of the corpus callosum evoked only the fast EPSP without inducing any types of IPSP in a majority of layer V pyramidal cells in the frontal cortex from SAMP10 [10]. When the intensity of stimulation was increased, most neurons induced epileptiform discharges. Taken together, a similar disinhibitory mechanism may cause hyperexcitability of AMG neurons in SAMP10 partially contributing to the age-related deficit in learning and memory of SAMP10.

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