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Immunohistochemical Study on the Distribution of Kv1.2 in Aged Rat Brain

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In the present study, we demonstrated age-related changes in Kv1.2 immunoreactivity in the rat brain for the first time.

Twelve adult (4 ~ 6 month old) and 15 aged (20 ~ 29 month old) Sprague-Dawley rats were examined in this study. Immunohistochemistry was performed in accordance with the free-floating method, and densitometric measurement using a NIH image program (Scion Image) determined the staining density.

In the cerebral cortex of aged rats, there was a significant increase in the number of Kv1.2-immunoreactive neurons in the cingulate cortex, infralimbic cortex and piriform cortex, compare to adult rats. In the hippocampal CA1-3 regions, moderate Kv1.2 immunoreactivity was found in the cell bodies and processes of some medium to large-sized neurons in aged rats. The intensity was increased in the cell bodies of Kv1.2-positive neurons in the amygdala of aged rats, whereas the number of immunoreactive neurons was not significantly increased. It was noteworthy that age-related changes in Kv1.2-immunoreactive neurons were prominent in the facial nuclei, raphe magnus nuclei, and pontine and medullary reticular formation.

Although the present study has not addressed multiple mechanisms contributing to neuronal degeneration during aging, the first demonstration of age-related changes in Kv1.2 immnuoreactivity may offer a comprehensive understanding of the pathophysiology of aging and age-related neurodegenerative diseases such as Alzheimer's disease.

Key words : Kv1.2 channel, Aging, Cerebral cortex, Hippocampus, Amygdala, Brainstem, Immunohistochemistry

Introduction

A proper understanding of many CNS diseases and age-related disorders requires identification of the ion channels underlying them and knowledge of their normal physiological roles. Recently, several studies have reported the possibility that reactive oxygen species alter ionic channel function (Matsuura and Shattock 1991, Ruppersburg et al. 1991). Modifications of Kv channel activity by reactive oxygen species would lead to drastic changes in the electrical excitability of neuronal cells and could easily explain a tendency to brain hyperexcitability or neuronal death. Duprat et al. (1995) showed that different types of Kv channels are differently modified by reactive oxygen species. Conforti et al. (2000) provided evidence of the O_2 sensitivity of native Kv1.2 α subunits of K^+ channels. Inwardly rectifying K^+ currents (K_{IR}) were markedly reduced in cells neighboring infarcts with maximal alteration at 3 d after infarct followed by a

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partial recovery (Koller et al. 2000). Although oxidative stress as well as amyloids may result in a potential decrement in blood flow and oxygen delivery to the brain during aging (Ajmani et al. 2000), very little is known about age-related changes in Kv1.2 expression in the whole brain.

Transient cerebral ischemia initiates a process of cellular events that lead to the delayed neuronal degeneration of several brain regions in both humans and animal models (Pulsinelli et al. 1982, Petito and Pulsinelli 1984). In recent years, accumulating evidence has indicated that many neurons undergo apoptosis after global or focal ischemia. Studies have shown that increased K^+ efflux might be a primary step leading to apoptosis. Post-ischemic injury may include increased extracellular K^+ ($[K^+]_e$), which is mediated initially by the opening of voltage-gated K^+ (Kv) channels and later ATP-dependent K^+ channels. The anoxic depolarization caused by elevated $[K^+]_e$ may result in the excessive release of neurotransmitters, in particular, glutamate, promoting further spatial spread of cellular depolarization, depletion of energy stores, and advancement of injury cascade (Lee et al. 2000). Given that impaired blood flow and associated decrements in oxygen delivery can produce neuronal damage during aging, it could be hypothesized that the properties of Kv1 channels could be changed with aging. In the previous study, we demonstrated post-ischemic changes of Kv1.2 channel distribution (Chung et al. 2001). Therefore, we examine the expression of Kv1.2 channel in the adult and aged rat brain by immunohistochemistry. Our results have revealed for the first time that immunoreactivity for Kv1.2 was increased in the cerebral cortex, hippocampus, amygdala and brainstem of aged rats.

Materials and Methods

Twelve adult (4 ~ 6 month old) and 15 aged (20 ~ 29 month old) Sprague-Dawley rats were examined in this study. The rats used in this study were treated in accordance with the 'Principles of Laboratory Animal Care' (NIH publication No. 86-23). The animals were perfused transcardially with cold phosphate buffered saline (PBS, 0.02 M, pH 7.4), and then with ice-cold 4% paraformaldehyde. Brains were cryoprotected in a series of cold sucrose solutions, and were cut at 40 μm in the coronal plane. The sections were incubated using the free-floating method for 48 ~ 72 hrs at 4°C in primary antiserum containing Triton X-100 (0.3%), bovine serum albumin (0.5 mg/mL) and normal goat serum (3 drops/10 mL), and polyclonal anti-Kv1.2 antibody (product No. 010, Alomone Labs, Jerusalem, Israel) Sections were visualized according to the avidin-biotin complex (ABC) method, using an ABC kit (Vectastain™, Vector Laboratories, Berlingame, CA), and then developed for peroxidase reactivity with 3, 3′-diaminobenzidine (DAB) (Sigma, St. Louis, MO).

A sample of sections was reacted without any primary antiserum, whereas a different sample was reacted with a primary antiserum that had been preincubated for 24 hours with control antigen peptides. No sections from both groups exhibited any of the immunoreactivity described in this report. Sections from each adult and aged group were stained together eliminating conflicts between different experimental conditions. Visual assessment and densitometric measurement using a NIH image program (Scion Image) determined the staining density. Student t-test was performed to investigate whether age-related changes were statistically significant ($*p < 0.05$).

Results

In the present study, increased expression of Kv1.2 was obvious in the cerebral cortex, hippocampal regions, amygdaloid complex and brainstem of aged rats

(Table 1). In the cerebral cortex, several Kv1.2-immunoreactive neurons with a long process were scattered in the control group (Fig. 1A). In aged rats, Kv1.2 immunoreactivity was found in the apical dendrites and the cell bodies of pyramidal cells (Fig. 1B). There was a significant increase in the number of Kv1.2-immunoreactive neurons in several regions of aged cerebral cortex, including the cingulate cortex, infralimbic cortex (Fig. 1C) and piriform cortex (Fig. 1D). In the hippocampal regions, the number and intensity of Kv1.2-immunoreactive neurons were also increased in aged rats (Fig. 2A-D). Moderate Kv1.2 immunoreactivity was found in the cell bodies and processes of some medium to large-sized neurons in the hippocampal CA1-3 regions (Fig. 2B) and in the polymorphic layer of the dentate gyrus (Fig. 2D) in aged rats. The intensity was increased in the cell bodies of

Table 1. Changes in mean densities of Kv1.2 immunoreactivity in several brain regions during aging

Area	Adult	Aged
Cerebral cortex	53.6±6.3	71±9.1*
Hippocampus		
CA1-3 region	44.2±3.6	52.8±4.5*
Dentate gyrus	46.3±5.6	60.2±4.1*
Septal Nucleus	61.4±4.3	75.0±6.7*
Amygdala	63.0±6.2	79.1±8.4*
Facial nucleus	69.5±5.4	83.1±7.1*
Raphe magnus nucleus	72.2±8.8	92.3±9.2*
Reticular formation	75.4±9.3	102±11.2*

Mean density is the sum of the gray values of all the pixels in the selection that was divided by the number of pixels within the selection. Values are the mean±standard deviations. Student's t-test was performed (*$p < 0.05$).

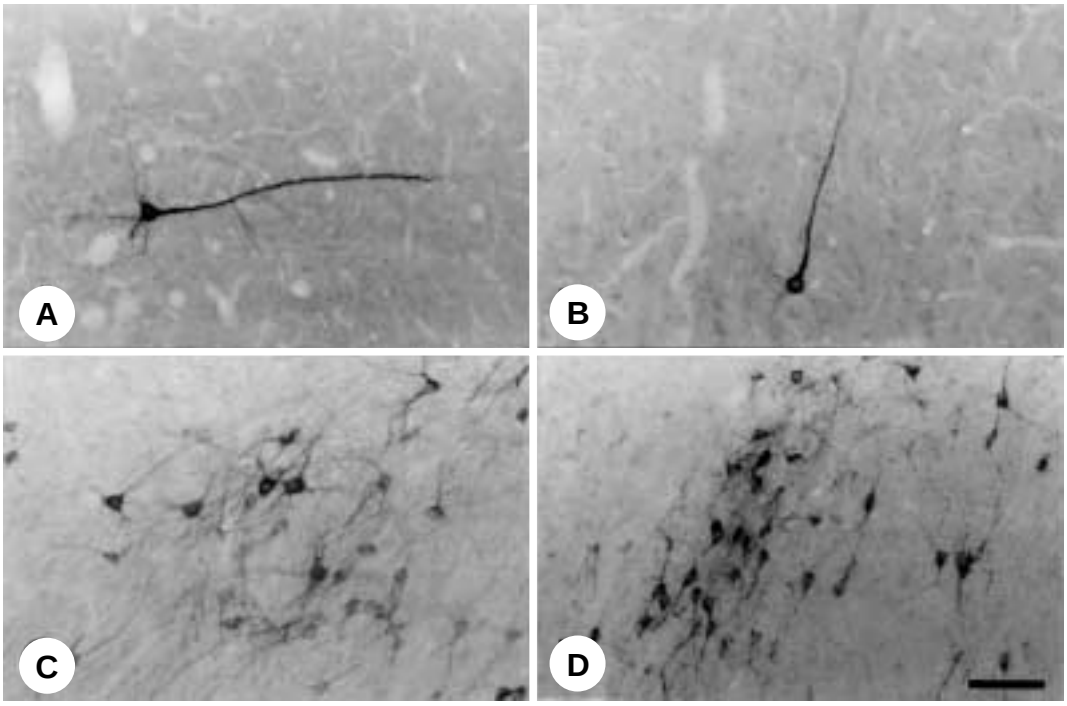

Fig. 1. Cellular Localizations of Kv1.2-immunoreactive neurons in the cerebral cortex of control (A) and aged (B, C, D) rats. In the control group, several Kv1.2-immunoreactive neurons with a long process were scattered in the cerebral cortex (A). In aged rats, Kv1.2 immunoreactivity was found in the apical dendrites and cell bodies of pyramidal cells (B). In aged cerebral cortex, there was a significant increase in the number of Kv1.2-immunoreactive neurons in several regions, including cingulate cortex, infralimbic cortex (C) and piriform cortex (D). Scale bar=50 μm

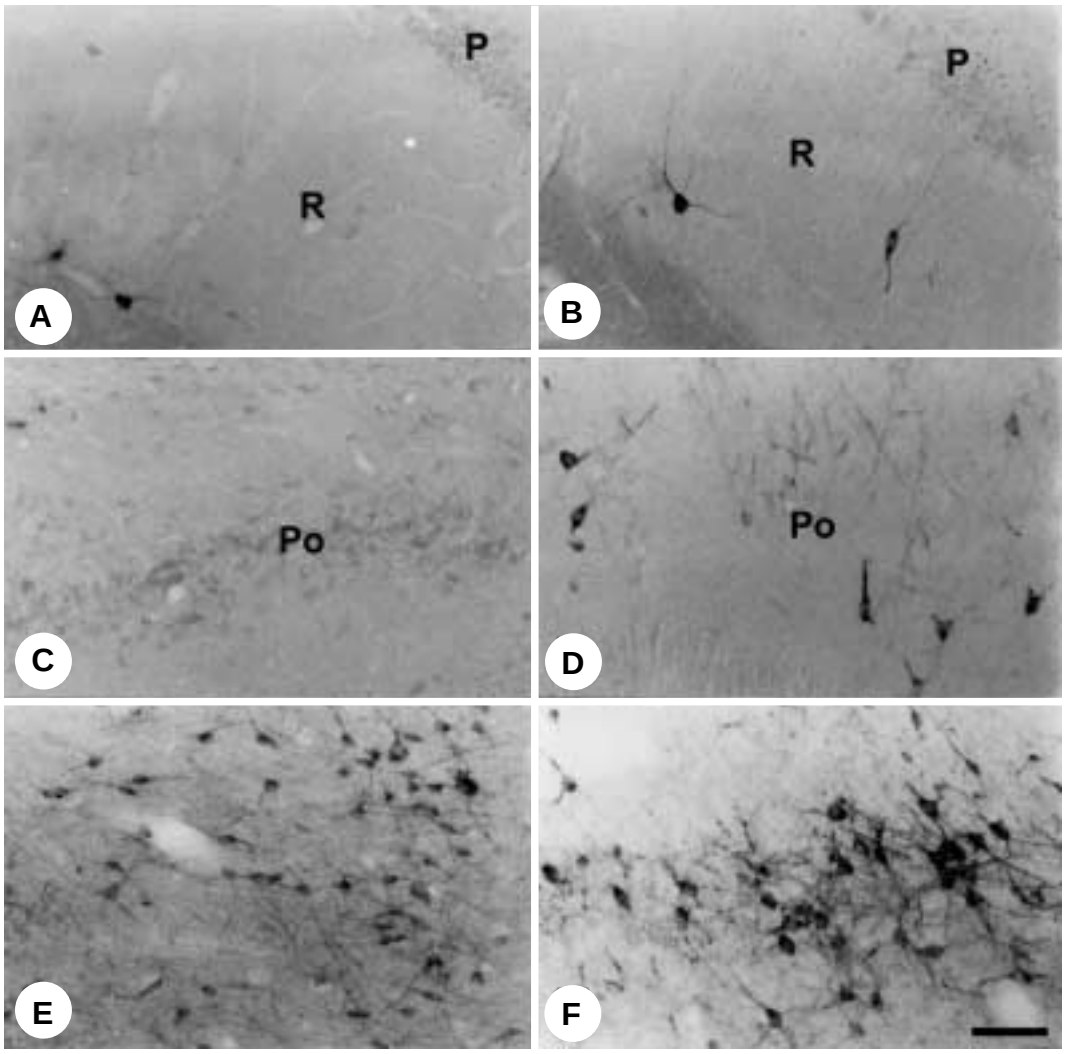

Fig. 2. Localizations of Kv1.2-immunoreactive neurons in CA1 subfield (A, B), dentate gyrus (C, D) and amygdala (E, F) of control (A, C, E) and aged (B, D, F) rats. In aged rats, moderate Kv1.2 immunoreactivity was found in the cell bodies and processes of some medium to large-sized neurons in the hippocampal CA1 region and in the polymorphic layer of the dentate gyrus, compared to control rats (A-D). The intensity was increased in the cell bodies of Kv1.2-positive neurons in the amygdala of aged rats, whereas the number of immunoreactive neurons was not significantly increased (E, F). P, pyramidal cell layer; Po, polymorphic layer; R, stratum radiatum. Scale bar=50 μm

Kv1.2-positive neurons in the amygdaloid nuclei of aged rats, whereas the number of immunoreactive neurons was not significantly increased (Fig. 2E, F). It was noteworthy that age-related changes in Kv1.2-immunoreactive neurons were prominent in the brainstem (Fig. 3). The number and intensity of Kv1.2-immunoreactive neurons were increased in the facial nuclei (Fig. 3A, B), raphe magnus nuclei (Fig. 3C, D), and pontine and medullary reticular formation of aged rats.

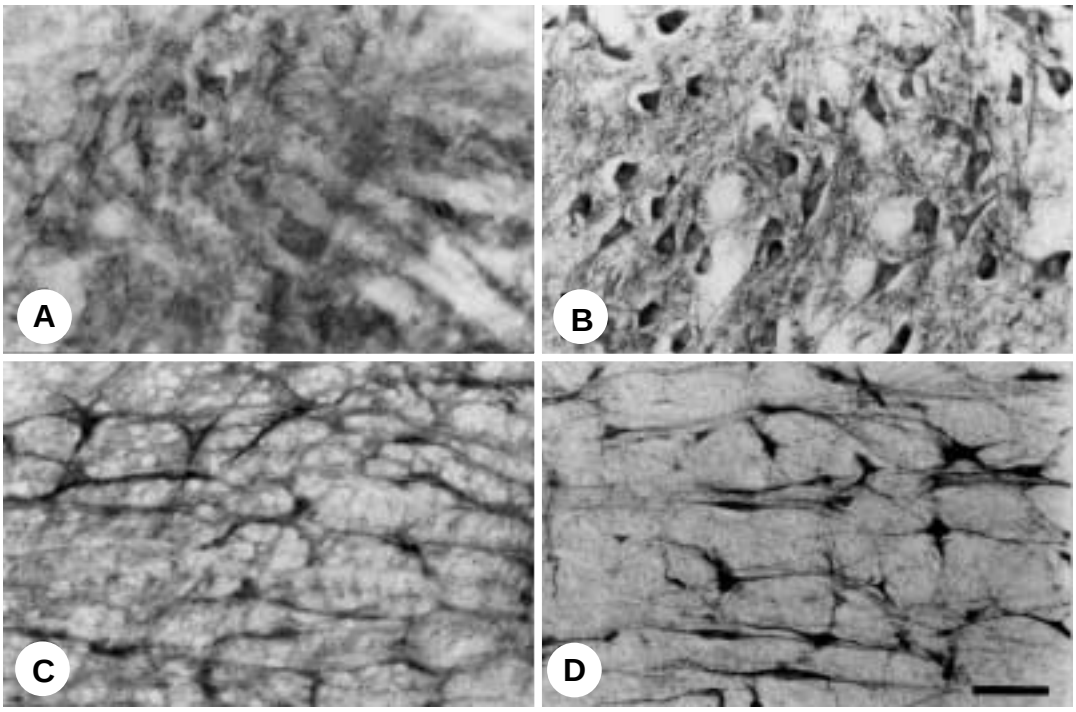

Fig. 3. Localizations of Kv1.2-immunoreactive neurons in the facial nuclei (A, B) and raphe magnus nuclei (C, D) of control (A, C) and aged (B, D) rats. It was noteworthy that age-related changes in Kv1.2-immunoreactive neurons were prominent in the brainstem. The number and intensity of Kv1.2-immunoreactive neurons were increased in the facial and raphe magnus nuclei of aged rats (B, D). Scale bar=50 μm

Discussion

In aged rats used in this study, impaired blood flow and associated decrements in oxygen delivery can produce neuronal damage in the central nervous system (Ajmani et al. 2000), which may be attributed to increased expression of Kv1.2. Aging produces a hemorheological decrement resulting in part from enhanced oxidative stress in old subjects. These changes can contribute to impaired cerebral blood flow, and influence the delivery of oxygen to the neurons associated with cognitive function. In older human subjects, impaired cerebral blood flow/oxygen delivery may be involved in a cascade of other changes related to Alzheimer's disease (Ajmani et al. 2000). These findings emphasize the importance of considering the normal age-dependent changes in blood hemorheology and blood flow in the etiology of Alzheimer's disease. The relationships shown to exist between hemorheology, blood flow, amyloids, oxidative stress, and cognitive function suggest that Kv1.2 channel may be one of the mechanisms operating in the complex pathology of aging and Alzheimer's disease.

Conforti and colleagues (1997) showed that the gene encoding the Kv1.2 subunit was selectively stimulated during prolonged exposure to hypoxia, and that the increased expression of the Kv1.2 subunit gene was correlated with an enhanced response to hypoxia. Their recent research provided evidence that

the K_{O2} channel in the O_2-sensitive $PC1_2$ clonal cell line is a K_v channel composed of Kv1.2 subunits (Conforti et al. 2000). In *Xenopus* oocytes, anoxia inhibited the K^+ current carried by Kv1.2 (Conforti et al. 2000). Since the Kv1.2 subunit is expressed in many different tissues including the nervous system, it might play a special role in neuronal cells with aging that is similar to mild hypoxia.

One of the more vulnerable neuronal populations resides in the CA1 region of hippocampus, a brain structure that is important for learning, memory and cognitive function (Zola Morgan et al. 1992). Electrophysiological studies have indicated that spontaneous firing rate decreases in CA1 region following ischemia (Imon et al. 1991) and the excitability of CA1 neurons is depressed (Urban et al. 1989). Several studies have demonstrated the potentiation of synaptic transmission and progressive suppression of intrinsic excitability in most CA1 neurons following severe forebrain ischemia (Urban et al. 1989, Gao et al. 1999). The diminished excitability, therefore, may be involved in the mechanisms of neuronal loss following ischemia (Gao et al. 1999). It has been shown that the decreased excitability in CA1 neurons during hypoxia in vitro is mainly due to an increase in potassium conductance (Leblond and Krnjevic 1989). It was consistent with our results that Kv1.2 expression was increased in some neurons in CA1 region of hippocampus. Therefore, the increment of Kv1.2 expression in CA1 region contributes not only to excitability changes but also to cell death during aging.

The overall results of the above localization study have demonstrated age-dependent alterations in the expression patterns of Kv1.2 in the cerebral cortex, hippocampus, amygdala, and brainstem, for the first time. Although the present study has not addressed multiple mechanisms contributing to neuronal cell death during aging, the first demonstration of age-related changes in Kv1.2 channel expression may offer a comprehensive understanding of the pathophysiology of aging and age-related neurodegenerative diseases such as Alzheimer's disease. The elucidation of the precise role of Kv channel subunits during aging will be a major topic for further study.

References

Ajmani RS, Metter EJ, Jaykumar R, Ingram DK, Spangler EL, Abugo OO, Rifkind JM : Hemodynamic changes during aging associated with cerebral blood flow and impaired cognitive function. Neurobiol Aging 21: 257-269, 2000.

Chung YH, Kim HS, Shin CM, Kim MJ, Cha CI : Immunohistochemical study on the distribution of voltage-gated K^+ channels in rat brain following transient focal ischemia. Neurosci Lett 308: 157-160, 2001.

Conforti L, Millhorn DE : Selective inhibition of a slow-inactivating voltage-dependent K^+ channel in rat PC12 cells by hypoxia. J Physiol 502: 293-305, 1997.

Conforti L, Bodi I, Nisbet JW, Millhorn DE: O_2-sensitive K^+ channels: role of the Kv1.2 -subunit in mediating the hypoxic response. J Physiol 524: 783-793, 2000.

Duprat F, Guillemare E, Romey G, Fink M, Lesage F, Lazdunski M, Honore E : Susceptibility of cloned K^+ channels to reactive oxygen species. Proc Natl Acad Sci USA 92: 11796 -11800, 1995.

Gao TM, Pulsinelli WA, Xu ZC : Changes in membrane properties of CA1 pyramidal neurons after transient forebrain ischemia in vivo. Neuroscience 90: 771-780, 1999.

Imon H, Mitani A, Andou Y, Arai T, Kataoka K : Delayed neuronal death is induced without post-ischemic hyperexcitability: continuous multiple-unit recording from ischemic CA1 neurons. J Cereb Blood Flow Metab 11: 819-823, 1991.

Koller H, Schroeter M, Jander S, Stoll G, Siebler M : Time course of inwardly rectifying K^+ current reduction in glial cells surrounding ischemic brain lesions. Brain Res 872: 194-198, 2000.

Leblond J, Krnjevic K : Hypoxic changes in hippocampal neurons. J Neurophysiol 62: 1-14, 1989.

Lee J-M, Grabb MC, Zipfel GJ, Choi DW : Brain tissue responses to ischemia. J Clin Invest 106: 723-731, 2000.

Matsuura H, Shattock MJ : Effects of oxidant stress on steady-state background currents in isolated ventricular myocytes.

Am J Physiol 261: H1358-H1365, 1991.

Petito CK, Pulsinelli WA : Delayed neuronal recovery and neuronal death in rat hippocampus following severe cerebral ischemia: possible relationship to abnormalities in neuronal processes. J Cereb Blood Flow Metab 4: 194-205, 1984.

Pulsinelli WA, Brierley JB, Plum F : Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11: 491-498, 1982.

Ruppersberg JP, Stocker M, Pongs O, Heinemann SH, Frank R, Koenen M : Regulation of fast inactivation of cloned mammalian IK_A channels by cysteine oxidation. Nature 352: 711-714, 1991.

Urban L, Neill KH, Crain BJ, Nadler JV, Somjen GG : Post-ischemic synaptic physiology in area CA1 of the gerbil hippocampus studied in vitro. J Neurosci 9: 3966-3975, 1989.

Zola Morgan S, Squire LR, Rempel NL, Clower RP, Amaral DG : Enduring memory impairment in monkeys after ischemic damage to the hippocampus. J Neurosci 7: 2582-2596, 1992.

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노화 흰쥐 뇌에서 Kv1.2의 분포에 관한 면역조직화학적 연구

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간추림 : 본 연구에서는 흰쥐 뇌에서 Kv1.2 면역염색성의 노화에 따른 변화를 규명하였다.

성숙 흰쥐와 노화 흰쥐를 사용하였고 면역조직화학법을 이용하여 염색한 후 영상분석을 실시하였다.

노화 흰쥐의 대뇌겉질에서, Kv1.2 면역염색성은 피라미드 세포의 가지돌기와 세포체에서 주로 관찰되었다. 띠겉질, 변연계아래 겉질 및 조롱박겉질 등의 대뇌겉질의 여러 부위에서 면역염색성을 나타내는 신경세포들의 수가 증가했다. 노화 흰쥐의 고유해마의 CA1 부위에서는 몇몇 중간 크기의 신경세포의 세포체와 돌기에서 중간정도의 Kv1.2 면역염색성을 보였다. 노화 흰쥐의 편도핵에서는 Kv1.2 면역염색성을 나타내는 신경세포의 세포체에서 염색정도가 증가한 반면, 염색된 신경세포의 수는 대조군과 비교하여 별다른 차이를 보이지 않았다. 노화에 따른 염색성의 증가가 가장 뚜렷한 부위는 뇌줄기였다. Kv1.2 면역염색성을 나타내는 신경세포의 수와 염색강도는 얼굴신경핵, 솔기핵, 다리뇌 및 숨뇌 그물체에서 증가하였다.

본 연구에서 노화에 따른 신경퇴행성 변화의 복합적인 기전에 대해서는 정확히 밝히지 못했지만, Kv1.2 채널 발현의 노화에 따른 변화에 대한 본 연구는 노화 및 노화와 관련된 신경퇴행성질환의 병태생리의 이해에 도움을 줄 것이라 사료된다.

찾아보기 낱말 : Kv1.2 채널, 노화, 대뇌겉질, 해마, 편도체, 뇌줄기, 면역조직화학

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