Effect of Aging in Bindings of Second Messengers in the Rat Brain

III. 11. Effect of Aging in Bindings of Second Messengers in the Rat Brain

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It is generally accepted that neurotransmitters play an important role in neuronal function and neurotransmission in the central nervous system (CNS). The process of aging can be characterized by a great number of physiological and biochemical modifications such as neuronal degeneration and alterations in neurotransmitter receptors. Several lines of evidence demonstrate that the age-related decline in cognitive functions such as memory and learning is closely linked to dysfunction of various neurotransmitter systems[1-5]. Therefore, the effect of aging on neurotransmitter systems is of great interest in regards to the specificity of age-related neuronal dysfunction and decline in cognitive function.

Many receptors for neurotransmitters in the CNS are well known to be coupled to the intracellular second messenger systems, the adenylate cyclase and phosphoinositide cycle systems. The two second messenger systems play a crucial role in mediating the actions of various neurotransmitters in the brain[6-8]. Recent studies suggested that a decrease in protein kinase C (PKC) activity in the phosphoinositide cycle system was found in the cortex and hippocampus of aged rats[9,10] and was closely associated with the impairment of spatial learning in the hippocampus[9]. On the other hand, Worley et al[11]. demonstrated that a reduction in adenylate cyclase activity was seen in the striatum and substantia nigra of aged rats. These observations are great interest in regard to any age-related decline in cognitive function. However, the role of second messenger alterations in aging processes is not fully understood. In the present study, therefore, we focused on major second messengers and analyzed the regional age-related changes of these bindings in the rat brain, using in vitro receptor autoradiography. For this purpose, [^{3}H]PDBu and [^{3}H]forskolin were used to label to protein kinase C (PKC) and adenylate cyclase, respectively.

Materials and Methods

Experimental animals: Male Fischer 344 rats, 3 weeks and 6, 12, 18 and 24 months of age, were used throughout the experiments. Animals were killed by decapitation: then brains were removed quickly, frozen in powdered dry ice, and stored at -80°C until assay.

Sagittal sections 12 mm in thickness were cut on a cryostat and thaw-mounted onto gelatin-coated cover slides. Adjacent sections stained with Cresyl violet were examined with a light microscope.

Receptor autoradiography

[^{3}H]PDBu binding

Autoradiographic localization of PDBu binding in the brain was performed as described previously[12,13]. Brain sections were incubated with 2.5 nM [^{3}H]PDBu (New England Nuclear, spec. act. 20.7 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.7) containing 100 mM NaCl and 1 mM NaCl for 60 min at 25°C. Following incubation, the sections were washed twice at 4°C for 2 min in the buffer and briefly rinsed in distilled water at 4°C. Non-specific binding was determined using 1 μM PDBu (Sigma).

[^{3}H]Forskolin binding

Autoradiographic distribution of forskolin binding was performed as described previously[14,15]. Brain sections were incubated with 10 nM [^{3}H]forskolin (New England Nuclear, spec. act. 33.0 Ci/mmol) in 50 mM Tris-HCl buffer containing 100 mM NaCl and 5 mM $MgCl_2$ for 10 min at 25°C. Following incubation, the sections were washed twice for 2 min at 4°C in the buffer. Non-specific binding was determined using 10 μM forskolin (Sigma).

All procedures were performed under subdued lighting. The sections were dried under a cold air stream and apposed to Hyperfilm-^{3}H (Amersham) for 7 days (PDBu binding) and 4 weeks (forskolin binding) in X-ray cassettes with a set of tritium standards. The optical density of the brain regions was measured by a computer-assisted image analyzer as described previously[14,15]. The relationship between optical density and radioactivity was obtained with reference to ^{3}H-microscales co-exposed with the tissue sections using a third-order polynomial function. Anatomical structures were verified by examination of Cresyl violet-stained sections. Binding assays were performed in duplicate. Values were expressed as mean± S.E. Statistical comparisons were determined using the ANOVA following by Dunnett's multiple comparison test. Each group contained five to seven animals.

Results

Regional age-related alterations of [^{3}H]PDBu and [^{3}H]forskolin bindings are summarized in Tables 1 and 2.

[^{3}H]PDBu binding

Regional distribution of [^{3}H]PDBu binding throughout the brain was relatively heterogeneous. In adult rats (6-month-old), the highest density of [^{3}H]PDBu binding was

noted in the molecular layer of cerebellum, hippocampus and neocortex. The striatum, nucleus accumbens, substantia nigra and thalamus also exhibited relatively high [^{3}H]PDBu binding. Other regions such as hypothalamus, midbrain and brain stem had a low grain density of [^{3}H]PDBu binding. In immature rats (3-week-old), the cerebellum showed a significant decrease in [^{3}H]PDBu binding as compared to that in adult animals. However, other regions exhibited no significant change in [^{3}H]PDBu binding. In contrast, no significant changes of [^{3}H]PDBu binding was observed in all brain areas in 12-, 18- and 24-months-old rats, as compared with 6-month-old rats.

[^{3}H]Forskolin binding

Autoradiographic localization of [^{3}H]forskolin binding in the rat brain was strikingly heterogeneous. In adult animals, the highest binding was noted in the striatum and nucleus accumbens followed by the hilus of the dentate gyrus, the molecular layer of the cerebellum, the hippocampal CA3 pyramidal layer and substantia nigra. Other regions showed relatively low [^{3}H]forskolin binding. In immature rats, a significant increase in [^{3}H]forskolin binding was observed in the neocortex, nucleus accumbens, thalamus and substantia nigra. Other regions revealed no significant changes in [^{3}H]forskolin binding. On the other hand, [^{3}H]forskolin binding was not significantly changed in the rat brain of 12-month-old rats. However, [^{3}H]forskolin binding in various brain regions showed a significant decline in 18-month-old rats. The age-related reduction was noted in the neocortex, hippocampal CA3 pyramidal cell layer, dentate gyrus, thalamus and molecular layer of the cerebellum. In 24-month-old rats, [^{3}H]forskolin binding showed significant decreases in various brain regions, as compared with 6-month-old animals.

Discussion

The present study provides evidence that adenylate cyclase system in the rat brain was more susceptible to aging processes than phosphoinositide cycle system. Furthermore, our autoradiographic results suggest that the change in the adenylate cyclase system is more pronounced than that in the phosphoinositide cycle system in immature rat brain. These findings suggest that the adenylate cyclase system is primarily affected in aging processes and this may lead to neurological deficits.

Aging in mammals is associated with striking changes in neurotransmission which may underlie the age-related deficits in psychomotor performance and cognitive function[1,16]. Numerous previous studies suggest that the binding sites of various neurotransmitters, such as acetylcholine, glutamate, dopamine, serotonin and noradrenaline, are altered in various brain regions during aging processes[17-22]. Furthermore, a selective loss of glutamate receptors and cholinergic neurons has been reported in the brain of Alzheimer's disease[23-26].

Thus, alterations in neurotransmitter receptors may play a key role not only in aging processes, but also in age-related neurodegenerative process such as Alzheimer' s disease.

Intracellular second messenger systems are well known to be crucial to various aspects of cellular functions. In particular, much evidence has emerged over the past decade to suggest that PKC in phosphoinositide cycle system is a widespread family of kinases responsible for neurotransmitter release, neuronal activity, synaptic plasticity, long-term potentiation (LTP), growth and differentiation[27-29]. Therefore, this enzyme is thought to play a major role in neuronal function. Previous investigations suggested an impaired PKC function in T lymphocytes of aged mice and a reduced phosphorylation of protein F_1, a specific endogenous PKC substrate in aged rat brain[30,31]. Friedman and Wang[10] also demonstrated the reduced PKC activity in aged rat brain. On the other hand, several neurotransmitters are known to stimulate the formation of cyclic-AMP by activation of adenylate cyclase[32]. Cyclic-AMP plays a role in expression or activation of ion channels and acetylcholine receptors[33,34]. Further, the cyclic-AMP cascade including adenylate cyclase is involved in learning, short-term memory and synaptic plasticity[35,36]. Nomura et al[8]. also suggest that basal activity of brain adenylate cyclase was significantly reduced in aged rats. Therefore, the adenylate cyclase system is rather likely implicated in aging processes as well as cognitive function.

In the present study, a significant decrease in [^{3}H]PDBu binding was observed only in the cerebellum of 3-week-old rats as compared with 6-month-old animals. In contrast, a significant increase in [^{3}H]forskolin binding was found in various brain regions, such as cerebral cortex, nucleus accumbens, thalamus and substantia nigra of 3 week-old animals. Interestingly, ontogenetic studies of PKC in the rat brain have demonstrated that this enzyme increases rapidly after birth and reaches the maximal level at about 4 weeks of postnatal age[37,38]. Therefore, no significant changes in [^{3}H]PDBu binding in most brain areas of 3-week-old rats observed in our study may reflect that the binding rapidly reaches near the adult level in the brain after birth. Although the detailed reason for this phenomenon is presently unclear, our finding may suggest the conspicuous differences in the developmental pattern between [^{3}H]PDBu and [^{3}H]forskolin binding in the brain after birth.

On the other hand, the present study also showed that age-related change in [^{3}H]forskolin binding was noted in various brain regions, whereas no significant alterations in [^{3}H]PDBu binding were observed in the brain of aged rats. In particular, the age-related reduction in [^{3}H]forskolin binding was observed in the cerebral cortex, striatum, nucleus accumbens, hippocampus such as CA3 pyramidal cell layer and dentate gyrus, thalamus and molecular layer of cerebellum. This finding seems to relate the finding that basal activity of adenylate cyclase is significantly decreased in aged rat brain. From these observations, we suggest that adenylate cyclase system in most brain regions may be susceptible to aging processes as compared with phosphoinositide cycle system. However, several subtypes of

PKC have been identified by protein purification and by cloning of cDNAs[39-42]. Therefore, it is necessary to investigate the regional pattern of each subtype of PKC for the further understanding of age-related changes in the brain using receptor autoradiographic and immunohistochemical techniques.

There is abundant evidence that several neurotransmitters, their marker enzymes and any neurotransmitter receptors are significantly reduced in brain tissue from Alzheimer's disease[23-26,43-47]. The dysfunction of intracellular second messenger systems is also known to occur in such age-related neurodegenerative disorders as Alzheimer's disease. Several studies have shown a marked reduction of PKC levels and the number of 1,4,5-trisphosphate(1P3) bindion in the neocortex and hippocampus of Alzheimer's disease patients[48,49]. Dewer et al50). also reported that [^{3}H]forskolin binding was significantly reduced in the cerebral cortex of Alzheimer's disease. These observations seem to suggest that the dysfunction of second messenger systems may reflect the impairment of cognitive function occurring not only in aged animals, but also in age-related neurodegenerative disorders such as Alzheimer's disease. Therefore, our finding may help to further elucidate the relationship between aging processes and cognitive function. However, the present study could not determine whether the observed changes in [^{3}H]PDBu and [^{3}H]forskolin bindings are due to changes in the affinity constant (Kd) or the number of binding sites (Bmax). Therefore, further studies are needed to investigate the precise biochemical mechanisms for our findings.

Conclusion

The present study demonstrates that adenylate cyclase system in the rat brain is more susceptible to aging processes than phosphoinositide cycle system. Furthermore, our results suggest that the changes in the adenylate cyclase system are more pronounced than that in the phosphoinositide cycle system in immature rat brain. These findings suggest that intracellular signal transduction is affected in aging processes and this may provide a new approach to age-related neurological deficits.

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Table 1. Regional age-associated changes in [^{3}H]PDBu binding in the rat brain.

Region	Age				
	3 weeks	6 months	12 months	18 months	24 months
Frontal cortex	852 ± 176	762 ± 71	801 ± 110	821 ± 91	732 ± 68
Parietal cortex	808 ± 204	895 ± 249	772 ± 103	794 ± 91	700 ± 74
Striatum	743 ± 134	622 ± 49	654 ± 42	741 ± 175	634 ± 55
Nucleus accumbens	785 ± 162	701 ± 112	725 ± 79	854 ± 313	776 ± 55
Hippocampus					
CA1 sector	960 ± 252	1091 ± 226	998 ± 125	1018 ± 66	891 ± 71
CA3 sector	896 ± 212	894 ± 80	885 ± 143	910 ± 159	875 ± 62
Dentate gyrus	888 ± 181	896 ± 56	906 ± 120	900 ± 95	901 ± 95
Thalamus	614 ± 140	501 ± 26	499 ± 85	529 ± 98	510 ± 43
Substantia nigra	727 ± 193	662 ± 112	602 ± 192	615 ± 137	668 ± 81
Cerebellum					
Molecular layer	1042 ± 120 *	1259 ± 90	1358 ± 133	1234 ± 123	1213 ± 94
Granule cell layer	410 ± 42 **	483 ± 58	465 ± 51	541 ± 70	477 ± 18

Optical density was coverted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means±S.E. *p<0.05, **p<0.01 vs 6 months old group (Dunnett's multiple range test). n=5-7

Table 2. Regional age-associated changes in [^{3}H]forskolin binding in the rat brain.

Region	Age				
	3 weeks	6 months	12 months	18 months	24 months
Frontal cortex	75 ± 7 **	56 ± 6	53 ± 5	46 ± 4 *	45 ± 9 *
Parietal cortex	78 ± 7 *	62 ± 11	63 ± 8	52 ± 11	54 ± 9
Striatum	269 ± 34	255 ± 17	236 ± 46	215 ± 13	201 ± 40 *
Nucleus accumbens	263 ± 23 *	222 ± 19	214 ± 25	205 ± 19	174 ± 32 **
Hippocampus					
CA1 sector	40 ± 9	33 ± 7	33 ± 7	30 ± 5	32 ± 8
CA3 sector	79 ± 8	73 ± 13	75 ± 10	59 ± 9	60 ± 16
CA3 pyramidal-cell layer	127 ± 14	134 ± 15	125 ± 15	105 ± 15 **	106 ± 19 **
Dentate gyrus	88 ± 11	80 ± 7	77 ± 5	71 ± 7	69 ± 8 *
Hilus	194 ± 19	183 ± 31	193 ± 20	178 ± 19	160 ± 22
Thalamus	101 ± 7 **	47 ± 7	47 ± 4	34 ± 8 *	35 ± 11 *
Substantia nigra	193 ± 41 **	118 ± 19	102 ± 15	99 ± 17	87 ± 14
Cerebellum					
Molecular layer	169 ± 20	161 ± 16	149 ± 8	142 ± 8 *	132 ± 8 *
Granule cell layer	50 ± 9	52 ± 12	53 ± 8	47 ± 9	45 ± 7

Optical density was coverted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means±S.E. *p<0.05, **p<0.01 vs 6 months old group (Dunnett's multiple range test). n=5-7.