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Effect of Vinconate Against Regional Age-Related Changes in the Gerbil Brain

TSUTOMU ARAKI,*†[1] YASUO KANAI,† HIROYUKI KATO,* KYUYA KOGURE,* KATSURO SHUTO† AND YUKIHARU ISHIDA†

*Department of Neurology, Institute of Brain Diseases, Tohoku University School of Medicine, 1-1 Seiryo-Machi, Sendai, Japan
†Pharmacological Research Laboratory, Research Laboratories, Tokyo Tanabe Co., Ltd., Tokyo, Japan

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ARAKI, T., Y. KANAI, H. KATO, K. KOGURE, K. SHUTO AND Y. ISHIDA. *Effect of vinconate against regional age-related changes in the gerbil brain.* PHARMACOL BIOCHEM BEHAV **44**(1) 17-25, 1993.—We investigated age-related changes in the binding sites of muscarinic acetylcholine, forskolin, adenosine 3',5'-cyclic monophosphate (cAMP), and of a voltage-dependent L-type calcium channel blocker in the gerbil brain using receptor autoradiography. [^{3}H]Quinuclidinyl benzilate (QNB), [^{3}H]forskolin, [^{3}H]cAMP, and [^{3}H]PN200-110 were used to label muscarinic receptors, adenylate cyclase, cAMP-dependent protein kinase, and L-type calcium channels, respectively. In middle-aged animals (16-month-old gerbils), [^{3}H]QNB, [^{3}H]PN200-110, [^{3}H]forskolin, and [^{3}H]cAMP binding sites were elevated in the hippocampal region compared with that of young gerbils (4 weeks old). Further, a significant elevation in [^{3}H]forskolin binding was seen in the nucleus accumbens. In contrast, [^{3}H]QNB, [^{3}H]PN200-110, and [^{3}H]forskolin binding sites were reduced in the cerebellum, neocortex and thalamus, and hypothalamus in middle-aged animals, respectively. [^{3}H]cAMP binding was not altered in other regions except for an elevation in the hippocampus. Thus, the age-related alterations in receptor binding may proceed by different mechanisms in various brain regions. Chronic vinconate treatment partly modulated the age-related alterations in [^{3}H]QNB, [^{3}H]forskolin, and [^{3}H]cAMP binding in the hippocampus, but not that of [^{3}H]PN200-110. Vinconate also regulated the age-related changes in [^{3}H]forskolin binding in the nucleus accumbens. These results indicate that the age-related alterations in the binding sites of muscarinic acetylcholine, forskolin, cAMP, and L-type calcium channel blocker occur in particular in the hippocampus. Further, they suggest that a novel vinca alkaloid derivative, vinconate, can partly modulate age-related changes in these binding sites.

Aging Second messengers Neurotransmitter Vinconate Calcium channel Receptor autoradiography Gerbil

NEUROTRANSMITTERS play an important role in brain neurotransmission and function. A number of experimental studies of neurotransmitter receptor systems in various brain regions have been reported (43,50). Recent evidence has demonstrated age-related changes in neurotransmitter systems in the mammalian brain (36,51). Previous studies have suggested that muscarinic cholinergic receptors were markedly reduced in all regions of the aged rat brain but that choline acetyltransferase (CAT) activity was not affected in these areas (26,32). In contrast, other studies have demonstrated an increase in muscarinic cholinergic acetylcholine binding sites in both rat and mouse aged brain (47,48). Thus, there are discrepancies regarding muscarinic receptors. Calcium ions also play critical roles in various neuronal functions. Calcium-dependent acetylcholine release is reduced in brain slices of aged animals (21). Therefore, age-related changes in calcium metabolism may affect neuronal function, especially neurotransmitter release.

Physiological evidence has indicated a functional link between intracellular second messengers and neuronal activities such as transmitter release and ion conductance (11,37,46). Therefore, the alteration of intracellular second messenger systems is of importance regarding the aging process. In particular, phosphatidylinositide-generated second messengers such as protein kinase C (PKC) and inositol 1,4,5-trisphosphate (IP_3) are thought to play a key role in neuronal transmembrane signal transduction (14,18,52). Deficits in the intracellular actions of second messengers are of paramount

[1] Requests for reprints should be addressed to Tsutomu Araki, Ph.D., Pharmacological Research Laboratory, Research Laboratories, Tokyo Tanabe Co., Ltd., 33-3 Akabanekita 2-chome, Kita-ku, Tokyo 115, Japan.

importance in such aged-related neurodegenerative disorders as Alzheimer's and Parkinson's diseases (16,38,54). Recent studies have shown a marked reduction of PKC levels and/or the number of IP_3 binding sites in the neocortex and hippocampus of Alzheimer's disease patients (14,54). These observations are of particular interest relative to the role of second messengers in aging as well as dementia.

The well-established intracellular second messenger adenosine 3',5'-cyclic monophosphate (cAMP) has a wide-ranging role in biologic systems, including that of a key regulator molecule in mammalian tissue (24). It is produced from ATP by the enzymatic activity of hormone- and transmitter-sensitive adenylate cyclase (25) and the activity in brain is higher than that in all other tissues (44). The diterpene plant derivative forskolin stimulates adenylate cyclase by interacting directly with its catalytic subunit to stimulate enzymatic activity (45). However, the age-related alteration in the binding sites of these second messengers during aging is poorly understood.

Vinca alkaloids are used to treat cerebrovascular diseases. They are thought to improve cerebral metabolism and circulation through increasing glucose and oxygen utilization or calcium antagonistic action (28,29). We recently reported that a novel vinca alkaloid derivative, vinconate, prevented ischemic brain damage after transient cerebral ischemia in experimental animals (6–8). Further, we found that vinconate modulated the postischemic alteration in the binding of second messengers such as PKC, IP_3, and forskolin (3). However, little is known about the action of vinca alkaloids against regional age-related changes in the binding sites of neurotransmitters and intracellular second messengers. In the present study, we focused upon muscarinic acetylcholine, forskolin, cAMP, and voltage-dependent L-type calcium channels, and analyzed regional age-related changes of these binding sites in the gerbil brain. Further, we examined the effect of chronic treatment with vinconate against the age-related alterations of these binding sites.

METHOD

Animals

Male Mongolian gerbils (Seiwa Experimental Animals, Fukuoka, Japan) weighing 30–115 g were allowed food and water ad lib 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 μm in thickness were cut on a cryostat and thaw mounted onto gelatin-coated slides. Adjacent sections were stained with cresyl violet and hematoxylin & eosin and anatomic structures were verified by comparing these stained sections with the gerbil brain atlas of Loskota et al. (33). Animals were divided into four groups: (1) 4 weeks old; (2) vinconate (Tokyo Tanabe Co., Ltd.) at a dose of 5 mg/kg was administered orally (PO) twice daily for 10 days before decapitation; (3) vinconate at a dose of 12.5 mg/kg was administered PO twice daily for 10 days before decapitation; (4) vehicle (distilled water) was administered PO twice daily for 10 days before decapitation. Gerbils in groups (2)–(4) were 16 months old. Each group contained six to nine gerbils.

Receptor Autoradiography

Muscarinic acetylcholine. Muscarinic cholinergic receptors were quantified using the radiolabeled antagonist [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, 41.5 Ci/mmol, Amersham Corp., Arlington Heights, IL) as reported previously (5). Sections were incubated with 1 nM [^{3}H]QNB in phosphate buffer (pH 7.4) for 90 min at room temperature. The sections were then washed in the buffer for 5 min at 4°C. Nonspecific binding was determined using 1 μmol unlabeled atropine (Sigma Chemical Co., St. Louis, MO).

Voltage-dependent L-type calcium channel blocker (PN200-110). Autoradiographic visualization of L-type calcium channels using PN200-110, a 1,4-dihydropyridine calcium antagonist, was performed according to the method of Cortes et al. (17) with minor modifications (5). Sections were incubated with 0.1 nM [^{3}H]PN200-110 (71.5 Ci/mmol, DuPont/New England Nuclear Corp., Newton, MA) in 170 mM Tris-HCl buffer (pH 7.7) for 60 min at room temperature. The slides were then washed in the buffer for 20 min at 4°C. Nonspecific binding was determined using 1 μM unlabeled nitrendipine (Sigma).

Forskolin. Forskolin binding sites were autoradiographically localized as described previously (2). Sections were incubated for 10 min at room temperature in the buffer (50 mM Tris-HCl pH 7.7, 100 mM NaCl, 5 mM $MgCl_2$) containing 10 nM [^{3}H]forskolin (40 Ci/mmol, DuPont/New England Nuclear). The sections were washed twice for 2 min at 4°C in the buffer. Nonspecific binding was determined using 10 μM unlabeled forskolin (Sigma).

cAMP. Autoradiographic distribution of cAMP binding sites was determined according to the method of Gundlach and Urosevic (25) with minor modifications (4). Sections were preincubated for 20 min at room temperature in Krebs-HEPES buffer (pH 7.4; 118 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 11 mM glucose, 25 mM HEPES). The sections were then incubated for 90 min at room temperature in the buffer containing 10 nM [^{3}H]cAMP (51 Ci/mmol, Amersham) and 1 mM 3-isobutyl-1-methyl-xanthine (IBMX). The sections were washed for 1 min at 4°C in the buffer and briefly rinsed in distilled water at 4°C. Nonspecific binding was determined using 10 μM unlabeled cAMP (Sigma).

All procedures were performed under subdued lighting. The sections were dried under a cold stream of air and apposed to Hyperfilm-^{3}H (Amersham) for 2–4 weeks in X-ray cassettes with a set of tritium standards. The optical density of the brain regions was measured using a computer-assisted image analyzer. The relationship between optical density and radioactivity was obtained with reference to the [^{3}H]microscale coexposed with the tissue sections. Binding assays were performed in duplicate. Statistical comparisons were made using Williams–Wilcoxon multiple-range test.

RESULTS

Histopathology

Representative photographs in the gerbil brains are shown in Fig. 1. Brains in all gerbils were morphologically intact.

Receptor Autoradiography

Regional age-related changes of [^{3}H]QNB, [^{3}H]PN200-110, [^{3}H]forskolin, and [^{3}H]cAMP binding sites are summarized in Tables 1–4. Representative autoradiographs are shown in Figs. 2 and 3.

[^{3}H]QNB binding. In young gerbils (4 weeks old), the highest [^{3}H]QNB binding was noticed in the nucleus accumbens, striatum, dentate gyrus, and hippocampal CA1 sector, followed by the frontal cortex and hippocampal CA3 sector.

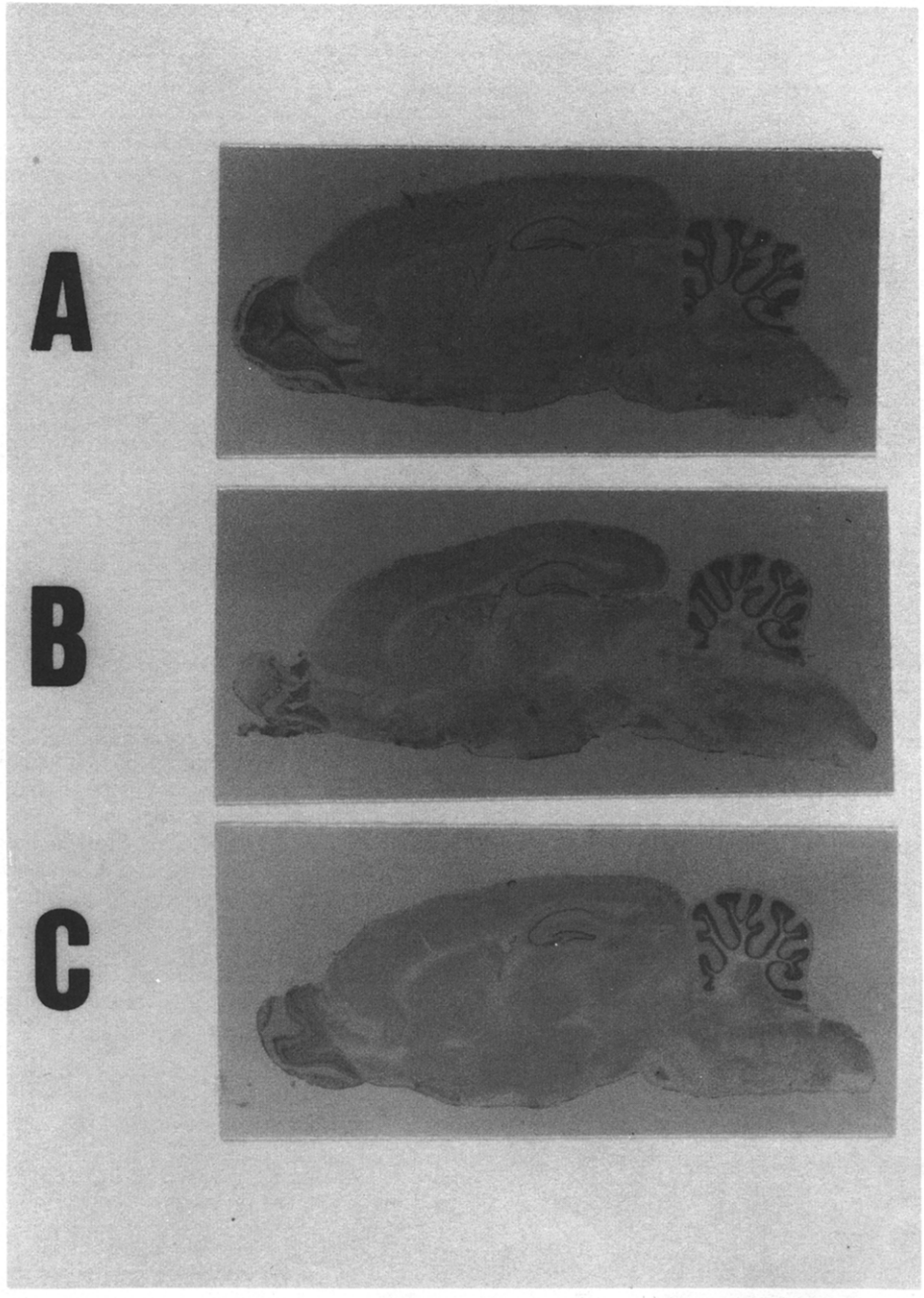

FIG. 1. Representative photographs of the gerbil brain. (A). Young gerbil (4 weeks old). (B). Vehicle-treated middle-aged gerbil (16 months old). (C). Vinconate (12.5 mg/kg, PO)-treated middle-aged gerbil (16 months old). Cresyl violet staining. Brains in all groups were morphologically intact.

However, [^{3}H]QNB binding was relatively low in the thalamus and hypothalamus and extremely low in the cerebellum. In vehicle-treated middle-aged animals (16 months old), the gray density of [^{3}H]QNB binding sites was relatively similar to that of young animals. However, a significant elevation in [^{3}H]QNB binding was seen in the hippocampal CA1 sector compared with young gerbils. In contrast, the cerebellum showed a significant reduction in [^{3}H]QNB binding. Chronic treatment with vinconate dose dependently modulated a significant elevation in [^{3}H]QNB binding sites in middle-aged animals. However, other regions showed no significant alteration in [^{3}H]QNB binding between vehicle- and vinconate-treated groups.

[^{3}H]PN200-110 binding. In young gerbils, the autoradiographic distribution of [^{3}H]PN200-110 binding in the brain was relatively heterogeneous, and the dentate gyrus and hippocampal CA3 sector exhibited high binding activity. The striatum, nucleus accumbens, thalamus, and neocortex also had relatively high [^{3}H]PN200-110 binding sites. However, [^{3}H]PN200-110 binding was relatively low in the hippocampal

TABLE 1

AGE-RELATED CHANGES IN [^{3}H]QNB BINDING IN THE GERBIL BRAIN

		16-Month-Old Gerbils		
			Vinconate (mg/kg)	
	4-Week-Old Gerbils	Vehicle	5	12.5
Frontal cortex	421 ± 9	396 ± 11	414 ± 14	389 ± 10
Striatum	578 ± 14	535 ± 28	573 ± 17	529 ± 11
Nucleus accumbens	584 ± 19	578 ± 36	618 ± 19	611 ± 28
Hippocampus				
CA1 sector	560 ± 17*	610 ± 12	568 ± 21*	550 ± 20*
CA3 sector	387 ± 15	421 ± 14	404 ± 17	387 ± 15
Dentate gyrus	562 ± 31	606 ± 17	578 ± 21	585 ± 18
Thalamus	188 ± 5	185 ± 11	203 ± 9	194 ± 12
Hypothalamus	195 ± 14	164 ± 9	182 ± 12	180 ± 7
Cerebellum	92 ± 7†	67 ± 6	80 ± 7	78 ± 7

Optical densities were converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means ± SEM. n = 7–9.

*$p < 0.05$, †$p < 0.01$ vs. vehicle-treated group (Williams-Wilcoxon multiple-range test).

CA1 sector and cerebellum and extremely low in the hypothalamus. In vehicle-treated middle-aged animals, the striatum and thalamus showed a significant reduction in [^{3}H]PN200-110 binding compared with young gerbils. In contrast, the hippocampal CA1 sector exhibited a significant elevation in [^{3}H]PN200-110 binding. In vinconate-treated middle-aged animals, the grain density of [^{3}H]PN200-110 binding in the brain was not different from that of the vehicle-treated middle-aged group.

[^{3}H]Forskolin binding. In young gerbils, [^{3}H]forskolin binding in the brain was strikingly heterogenous and the highest binding was noted in the striatum and nucleus accumbens, followed by the hilus of the dentate gyrus, the molecular layer of cerebellum, and the substantia nigra. Other regions showed relatively low [^{3}H]forskolin binding. In vehicle-treated middle-aged animals, a significant reduction in [^{3}H]forskolin binding was found in the hypothalamus, whereas a significant elevation in the binding was noted in the nucleus accumbens and hilus of dentate gyrus compared with young gerbils. Other regions showed no significant alteration in [^{3}H]forskolin binding. Chronic treatment with vinconate at a dose of 5 mg/kg did not significantly affect [^{3}H]forskolin binding in all regions compared with vehicle-treated middle-aged animals. In contrast, chronic treatment with vinconate at the higher dose modulated the elevation in [^{3}H]forskolin binding in the nucleus accumbens and hilus of the dentate gyrus. Other regions showed no significant changes in [^{3}H]forskolin binding in vinconate-treated middle-aged animals.

TABLE 2

AGE-RELATED CHANGES IN [^{3}H]PN200-110 BINDING IN THE GERBIL BRAIN

		16-Month-Old Gerbils		
			Vinconate (mg/kg)	
	4-Week-Old Gerbils	Vehicle	5	12.5
Frontal cortex	14 ± 1	14 ± 1	13 ± 2	15 ± 2
Striatum	17 ± 1*	14 ± 1	16 ± 1	15 ± 1
Nucleus accumbens	17 ± 1	15 ± 1	16 ± 2	16 ± 2
Hippocampus				
CA1 sector	9 ± 1†	15 ± 2	11 ± 2	13 ± 2
CA3 sector	24 ± 1	23 ± 2	24 ± 2	22 ± 2
Dentate gyrus	37 ± 4	39 ± 4	36 ± 2	35 ± 3
Thalamus	17 ± 2*	14 ± 1	15 ± 1	15 ± 2
Hypothalamus	1 ± 0	1 ± 1	1 ± 1	4 ± 1
Cerebellum	6 ± 1	5 ± 1	4 ± 1	5 ± 1

Optical densities were converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means ± SEM. n = 6–9.

*$p < 0.05$, †$p < 0.01$ vs. vehicle-treated group (Williams-Wilcoxon multiple-range test).

TABLE 3

AGE-RELATED CHANGES IN [^{3}H]FORSKOLIN BINDING IN THE GERBIL BRAIN

		16-Month-Old Gerbils		
			Vinconate (mg/kg)	
	4-Week-Old Gerbils	Vehicle	5	12.5
Frontal cortex	58 ± 2	54 ± 4	55 ± 4	48 ± 3
Striatum	587 ± 20	555 ± 19	537 ± 14	508 ± 17
Nucleus accumbens	505 ± 21*	577 ± 21	573 ± 17	473 ± 31*
Hippocampus				
CA1 sector	51 ± 2	47 ± 3	51 ± 7	42 ± 3
CA3 sector	93 ± 5	88 ± 9	85 ± 8	72 ± 4
Dentate gyrus	90 ± 4	93 ± 5	87 ± 8	82 ± 2
Hilus	200 ± 8†	259 ± 15	251 ± 17	214 ± 18†
Thalamus	60 ± 2	53 ± 3	57 ± 3	47 ± 3
Hypothalamus	64 ± 25*	43 ± 3	48 ± 5	38 ± 4
Cerebellum				
Average	76 ± 3	72 ± 2	73 ± 5	63 ± 3
Molecular layer	120 ± 3	127 ± 6	126 ± 8	111 ± 6

Optical densities were converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means ± SEM. n = 7–9.

*$p < 0.05$, †$p < 0.01$ vs. vehicle-treated group (Williams-Wilcoxon multiple-range test).

[^{3}H]cAMP binding. In young gerbils, [^{3}H]cAMP binding was seen in the dentate gyrus and hippocampal CA3 sector including the pyramidal neurons. The hippocampal CA1 pyramidal cell layer also exhibited a high number of [^{3}H]cAMP binding sites. Further, other regions also had relatively high amounts of [^{3}H]cAMP binding. In vehicle-treated middle-aged animals, the hippocampal CA3 sector and dentate gyrus had significantly elevated [^{3}H]cAMP binding compared with young gerbils. However, age-related alterations were not found in other regions. Chronic treatment with vinconate (12.5 mg/kg) modulated the elevation in [^{3}H]cAMP binding only in the dentate gyrus.

DISCUSSION

The present study demonstrated that age-related alterations in [^{3}H]QNB, [^{3}H]PN200-110, [^{3}H]forskolin, and [^{3}H]cAMP binding sites were found among various brain regions, especially in the hippocampus. Further, they suggested that a novel vinca alkaloid derivative, vinconate, partly modulated the age-related alteration in the binding sites of acetylcholine, forskolin, and cAMP in the hippocampus, whereas it failed to regulate the age-related change in the binding of voltage-dependent L-type calcium channel blocker.

Several studies have suggested that age-associated alterations in cognitive processes, in particular in learning and

TABLE 4

AGE-RELATED CHANGES IN [^{3}H]AMP BINDING IN THE GERBIL BRAIN

		16-Month-Old Gerbils		
			Vinconate (mg/kg)	
	4-Week-Old Gerbils	Vehicle	5	12.5
Frontal cortex	217 ± 13	253 ± 25	217 ± 17	241 ± 28
Striatum	144 ± 9	162 ± 16	150 ± 6	166 ± 15
Nucleus accumbens	217 ± 21	295 ± 41	217 ± 22	257 ± 17
Hippocampus				
CA1 sector	142 ± 7	172 ± 15	169 ± 14	191 ± 22
CA3 sector	271 ± 8†	329 ± 16	306 ± 12	315 ± 18
Dentate gyrus	308 ± 20†	376 ± 7	346 ± 10	336 ± 13*
Thalamus	140 ± 6	145 ± 15	149 ± 12	166 ± 14
Hypothalamus	222 ± 34	232 ± 41	181 ± 10	225 ± 23
Cerebellum	135 ± 6	135 ± 25	129 ± 16	137 ± 18

Optical densities were converted to fmol/mg tissue using [^{3}H]microscales. Values are expressed as means ± SEM. n = 7–9.

*$p < 0.05$ †$p < 0.01$, vs. vehicle-treated group (Williams-Wilcoxon multiple-range test).

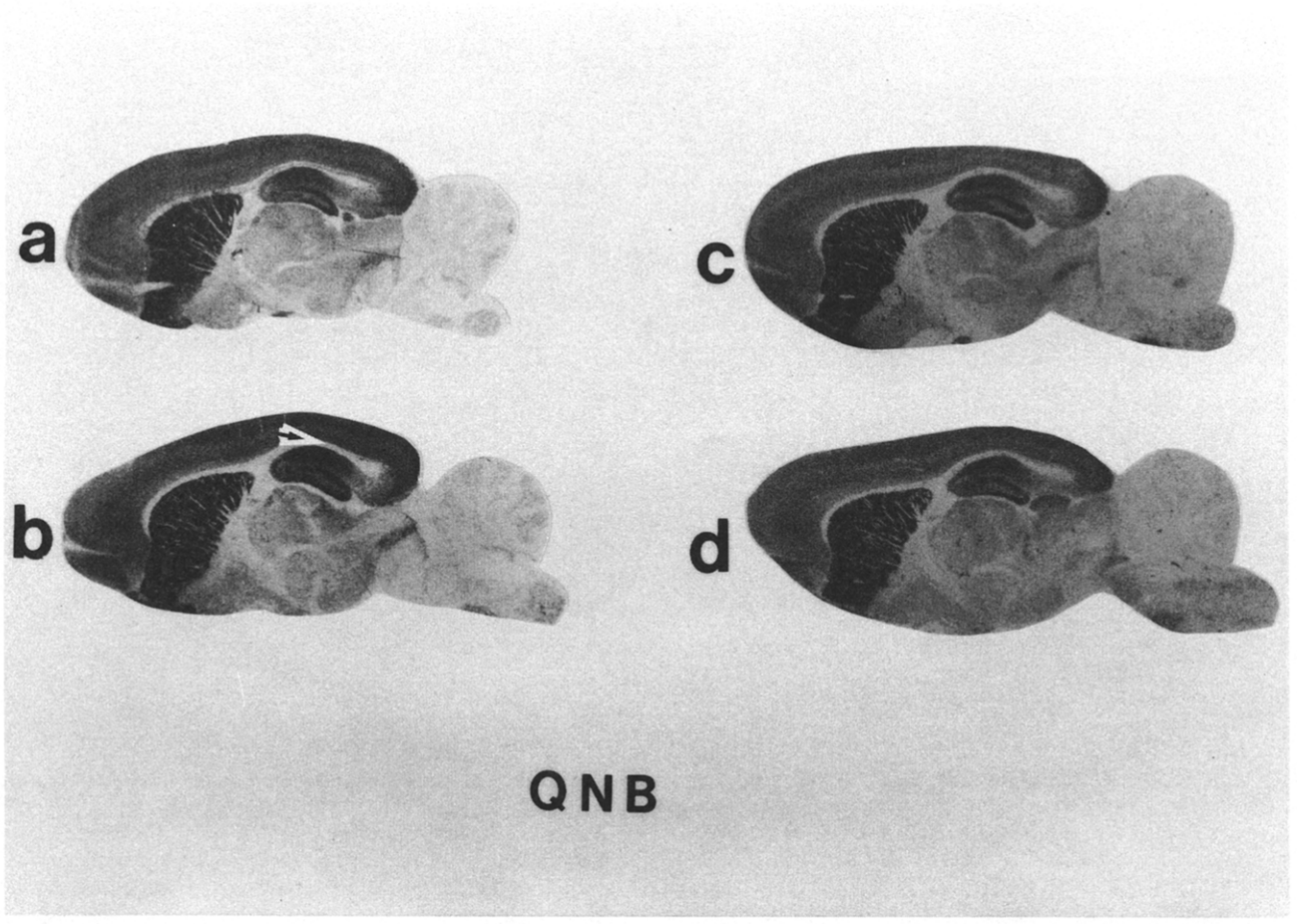

FIG. 2. Representative autoradiograms of [^{3}H]quinuclidinyl benzilate (QNB) binding sites in the gerbil brain. (a). Young gerbil. (b). Vehicle-treated middle-aged gerbil. (c). Vinconate (5 mg/kg, PO)-treated middle-aged gerbil. (d). Vinconate (12.5 mg/kg, PO)-treated middle-aged gerbil. A significant elevation in [^{3}H]QNB binding sites was noted in the hippocampal CA1 sector in the vehicle-treated middle-aged group compared with young gerbils (b, arrowhead). Vinconate dose dependently modulated the elevation in [^{3}H]QNB binding in the hippocampal CA1 sector (c,d).

memory, occur in experimental animals (19,41,42). In particular, the hippocampus plays a key role in cognitive function (10,34). The impairments in learning and memory in the aged rat are associated with an age-dependent decline of cholinergic function in the forebrain (12). Therefore, alterations in muscarinic acetylcholine receptors may reflect the impairment of brain function in aged animals. Also, altered calcium homeostasis is an important contributing factor in the expression of a number of neuronal functions altered during aging, such as neurotransmitter release, enzymatic functions, and transport systems (22). The brain is particularly rich in high-affinity voltage-dependent L-type calcium channel binding sites associated with neuronal elements (35). Therefore, an alteration in the ability of neurons to allow calcium entry may be responsible for the decline in neurotransmitter release observed in various brain areas during aging (23).

The present study demonstrated that [^{3}H]QNB binding was significantly increased in the hippocampal CA1 sector of middle-aged gerbils compared with young animals. In contrast, the cerebellum exhibited a significant reduction in [^{3}H]QNB binding sites in middle-aged animals. A previous study has shown an increase in [^{3}H]QNB binding in the hippocampus of aged mice (48). Springer et al. (47) also demonstrated a significant increase in [^{3}H]QNB binding in the hippocampal CA1 sector and dentate gyrus in aged rats. Further, they suggested that the increased [^{3}H]QNB binding in the hippocampus is upregulated to compensate for decreases in presynaptic cholinergic activity. Our results partly agree with theirs. On the other hand, the reason for the decreased [^{3}H]QNB binding in the cerebellum of middle-aged animals is at present unclear. However, this phenomenon may be, at least in part, correlated with previous reports in which an age-related decline in the number of [^{3}H]QNB binding sites was observed in aged rats (26,32). Our results also suggested that [^{3}H]PN200-110 binding was significantly decreased in the striatum and thalamus of middle-aged gerbils, whereas the binding was significantly increased in the hippocampal CA1 sector. Govoni et al. (23) previously reported that the number of nitrendipine (a voltage-dependent calcium channel blocker) binding sites increased in the aged rat brain, but the affinity of these binding sites declined nearly 50%. On the other hand, Battaini et al. (13) reported an increase in [^{3}H]verapamil (a voltage-dependent calcium antagonist) binding sites in the aged rat brain without any change in its affinity. These observations suggest that calcium channels are altered during aging. The present results are, at least in part, consistent with the findings

of Battaini et al. (13) and Govoni et al. (23). Although the reason for the decreased [^{3}H]PN200-110 binding sites in the striatum and thalamus is not clear, this phenomenon suggests that age-related alterations in the voltage-dependent calcium channel binding sites may be processed by different mechanisms in various brain regions.

Cellular responses to neurotransmitter receptor activity depend upon the integrity of coupling the recognition sites to their associated signal transduction mechanisms including intracellular second messenger systems. The two major second messenger systems in the brain are the generation of cAMP by the enzyme adenylate cyclase or by the phosphoinositide cycle, whereby hydrolysis of membrane lipids activates certain phosphorylating enzymes and mobilizes intracellular calcium (31,53). The binding sites of [^{3}H]forskolin to adenylate cyclase have been mapped autoradiographically in mammalian brain and exhibited a markedly heterogenous distribution (20). cAMP also acts as an intracellular second messenger to activate a specific protein kinase. Throughout the mammalian brain, [^{3}H]cAMP binding sites are also present (25). The distribution of [^{3}H]cAMP binding presumably reflects the regional distribution of protein kinase regulatory subunits that are membrane bound cAMP dependent and shows the ubiquity of cAMP-mediated transduction systems (25). It has been suggested that the basal activity of adenylate cyclase is elevated in the brains of senescent animals (49). On the other hand, Nomura et al. (39) reported that the basal activity of adenylate cyclase in the aged rat brain was significantly lower than that in the adult rat brain. These inconsistencies may be a consequence of different experimental conditions. Our autoradiographical data show an elevation in [^{3}H]forskolin and [^{3}H]cAMP binding sites in the hippocampus of middle-aged gerbils. These findings are of interest relative to the age-related impairment of cognitive functions. A significant increase in [^{3}H]forskolin binding sites of middle-aged gerbils was also seen in the nucleus accumbens. Further, the nucleus

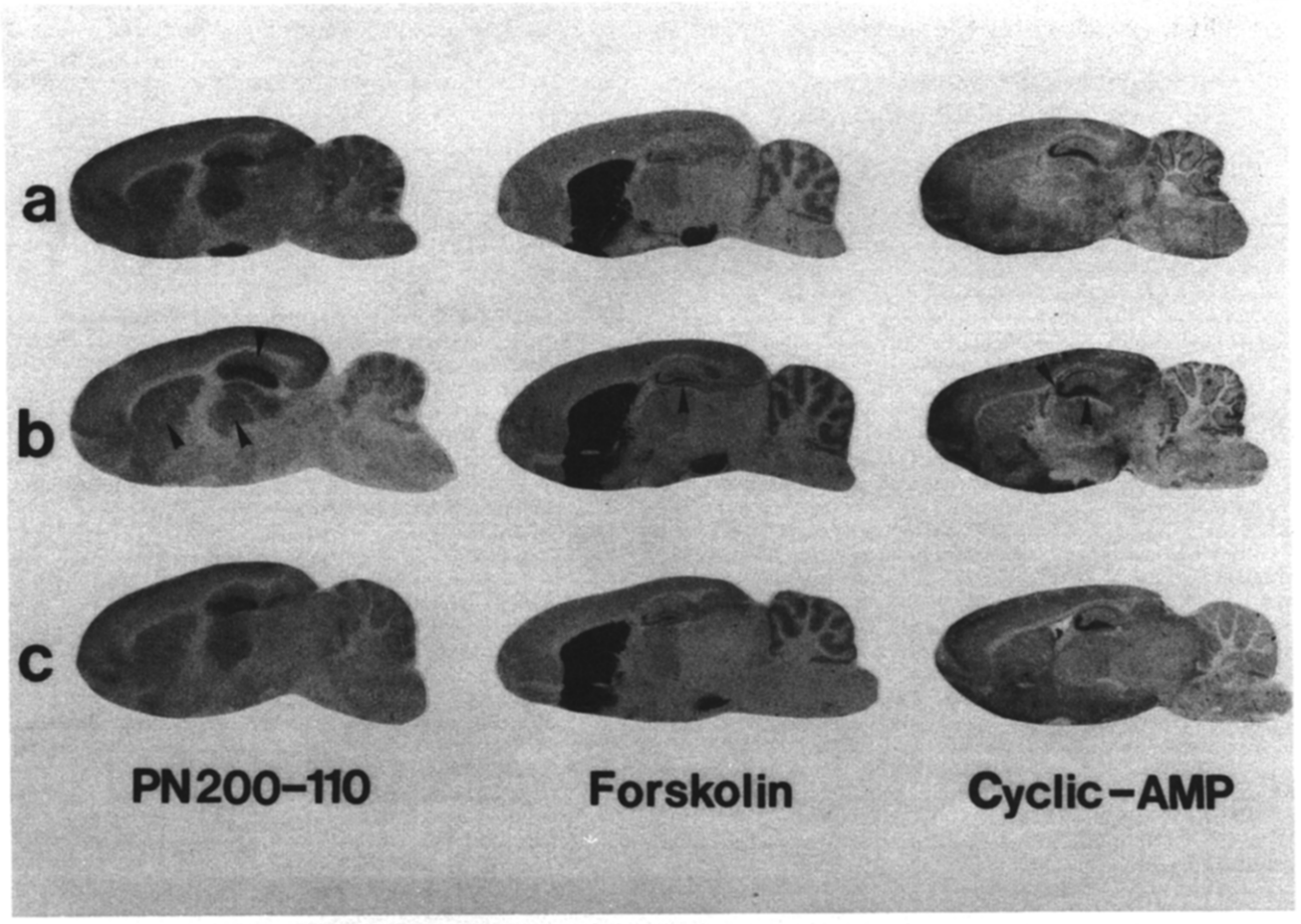

FIG. 3. Representative autoradiograms of [^{3}H]PN200-110, [^{3}H]forskolin, and [^{3}H]cyclic adenosine monophosphate (cAMP) binding sites in the gerbil brain. (a). Young gerbil. (b). Vehicle-treated middle-aged gerbil. (c). Vinconate (12.5 mg/kg, PO)-treated middle-aged gerbil. A significant reduction in [^{3}H]PN200-110 binding was noted in the striatum and thalamus in the vehicle-treated middle-aged group compared with young gerbils (b, arrowheads). In contrast, a significant elevation in [^{3}H]PN200-110 binding was seen in the hippocampal CA1 sector (b,arrowhead). Chronic vinconate treatment showed no significant alterations in [^{3}H]PN200-110 binding in the above sites (c). A significant elevation in [^{3}H]forskolin binding was observed in the hilus of the dentate gyrus in the vehicle-treated middle-aged group compared with that of young gerbils (b, arrowhead). Chronic vinconate treatment modulated the elevation in [^{3}H]forskolin binding in the hilus (c). A significant elevation in [^{3}H]cAMP binding was noted in the hippocampal CA3 sector and dentate gyrus in the vehicle-treated middle-aged group compared with that of young gerbils (b, arrowheads). Chronic vinconate treatment regulated the significant elevation in [^{3}H]cAMP binding in the dentate gyrus (c).

accumbens exhibited a high grain density of [^{3}H]QNB and [^{3}H]cAMP binding. Therefore, the nucleus accumbens as well as the hippocampus may play a role in the regulation of emotion and cognition.

Several neurotransmitters stimulate the formation of cAMP by activation of adenylate cyclase (40). Many of the specific receptors to which neurotransmitters bind are a component of the adenylate cyclase system. Of interest is that cAMP plays a role in expression or activation of ion channels and acetylcholine receptors (9,27). Further, it has been suggested that the cAMP cascade, including the adenylate cyclase system, plays a key role in learning, short-term memory, and synaptic plasticity (15,55). Therefore, this study indicates that age-related changes in the adenylate cyclase system play an important role in aging processes as well as cognitive functions.

A recent study has demonstrated that vinconate has an antiamnestic effect on basal forebrain-lesion-induced amnesia by ameliorating the dysfunction in cholinergic neurons (30). We recently reported that vinconate can modulate age-related alterations in the binding sites of PKC and IP_3 (1). This study also showed that chronic treatment with vinconate dose dependently modulated the age-related alterations in the binding of muscarinic acetylcholine in the hippocampal CA1 sector, whereas it failed to regulate age-related changes of voltage-dependent L-type calcium channel binding sites in the same field. Further, the results suggested that vinconate partly modulated age-related alterations in the binding sites of [^{3}H]forskolin and [^{3}H]cAMP in the nucleus accumbens and hippocampus. Especially, this effect was seen in the dentate gyrus including the hilus. Thus, an effect of vinconate in middle-aged gerbils was noted in the hippocampus, which plays an important role in cognitive function. Although the precise mechanisms for the action of vinconate against age-related alterations remain unclear, it seems to be partly mediated via the modulation of the cholinergic and adenylate cyclase systems.

In summary, the present study demonstrated age-related alterations in the binding sites of muscarinic acetylcholine, forskolin, cAMP, and a voltage-dependent L-type calcium channel blocker in various brain regions. This phenomenon was especially prevalent in the hippocampus of middle-aged gerbils. Further, it is suggested that vinconate partly modulates age-related alterations in the binding sites of acetylcholine, forskolin, and cAMP in the hippocampus, whereas it cannot regulate age-related change in the binding of voltage-dependent L-type calcium channel blocker. These results suggest that vinconate may partly modulate via regulating the cholinergic and adenylate cyclase systems in middle-aged gerbils. Further studies should be performed to investigate the precise mechanisms for such effects.

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