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Age-related Changes in [^{3}H]Nimodipine and [^{3}H]Rolipram Binding in the Rat Brain

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Abstract

Ageing is associated with changes in neurotransmission which might be correlated with abnormal calcium metabolism. Because there is evidence that nimodipine can enhance the learning abilities of ageing animals and rolipram can enhance the excitability of neurons, providing a functional basis for cognition-enhancing activity, age-related alterations in the binding of voltage-dependent L-type calcium channels and calcium/calmodulin-independent cyclic adenosine monophosphate-selective phosphodiesterase (cyclic-AMP PDE) were studied in 3-week- and 6-, 12-, 18- and 24-month-old Fisher 344 rats by use of receptor autoradiography.

[^{3}H]Nimodipine and [^{3}H]rolipram were used to label the voltage-dependent L-type calcium channels and calcium/calmodulin-independent cyclic-AMP PDE, respectively. [^{3}H]Nimodipine binding showed no obvious change in all brain areas of 12- and 18-month-old rats, as compared with 6-month-old animals. In 24-month-old rats, however, [^{3}H]nimodipine binding increased significantly in the striatum and hippocampal CA3 sector. In contrast, [^{3}H]rolipram binding showed no significant change in most brain areas during ageing, except for a transient change only in the hippocampal CA1 sector of 12-month-old animals. [^{3}H]Nimodipine and [^{3}H]rolipram binding showed a significant increase in some brain areas of 3-week-old rats compared with 6-month-old animals.

The results indicate that in rats voltage-dependent L-type calcium channels are more susceptible to ageing processes than calcium/calmodulin-independent cyclic-AMP PDE. Our data also demonstrate that voltage-dependent L-type calcium channels and calcium/calmodulin-independent cyclic-AMP PDE might play roles in developmental processes. These findings might help further elucidation of the relationship between age-related neurological deficits and behavioural pharmacology including cognitive function.

Nimodipine is a potent voltage-dependent L-type calcium channel antagonist which has a high affinity for the central nervous system and passes the blood-brain barrier rapidly (Scriabine et al 1989). Several lines of evidence have demonstrated that nimodipine can enhance the learning abilities of ageing rabbits, rats and monkeys (Deyo et al 1989; Sandin et al 1990; Straube et al 1990; Levere & Walker 1991). This drug has also been reported to be effective for the treatment of age-related disorders such as senile dementia (Tollefson 1990). There has, furthermore, been growing interest in the application of nimodipine as a protective agent against ischaemic brain damage (Uematsu et al 1991; Roda et al 1995). From these observations it is conceivable that nimodipine might have a role in the treatment of age-related neurological deficits and cognitive dysfunction. Little is, however, known about changes of [^{3}H]nimodipine binding in the ageing process.

Rolipram, known to be an antidepressant, inhibits a calcium/calmodulin-independent cyclic adenosine monophosphate-selective phosphodiesterase (cyclic-AMP PDE) isozyme (Kariya & Dage 1988), leading to an increase in brain cyclic-AMP levels (Schneider 1984). Regional distributions for [^{3}H]rolipram binding sites have been quantified and visualized in the brain by receptor autoradiography (Kaulen et al 1989). A recent study suggests that the PDE inhibitor denbufylline can enhance the excitability of neurons, providing a functional basis for cognition-enhancing activity (Nicholson et al 1991). The calcium/calmodulin-independent cyclic-AMP PDE might therefore, play a role in modulation of brain function, although the role of the cyclic-AMP PDE in ageing process is not fully understood.

In this study we have, therefore, used receptor autoradiography to investigate age-related changes in voltage-dependent L-type calcium channels and calcium/calmodulin-independent cyclic-AMP PDE in aged rat brain.

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Materials and Methods

Subjects

Male Fisher 344 rats, 3 weeks and 6, 12, 18 and 24 months old, were used. The animals were lightly anaesthetized with ether and then killed by decapitation. The brains were removed immediately, frozen in powdered dry ice, and stored at −80°C until assay. Sagittal sections 12 μm thick were cut on a cryostat and thaw-mounted on to gelatin-coated cover slides. Adjacent sections stained with cresyl violet were examined with a light-microscope. Each group contained five to seven rats.

Receptor autoradiography

[^{3}H]Nimodipine binding. Autoradiographic localization of voltage-dependent L-type calcium channels was detected using [^{3}H]nimodipine by the methods of Bellemann et al (1983) with minor modifications. Brain sections were pre-incubated for 30 min at room temperature in 50 mM Tris-HCl buffer (pH 7·4) containing 150 mM NaCl and 1 mM $CaCl_2$. The sections were then incubated with 1·5 nM [^{3}H]nimodipine (specific activity, 152 Ci mmol^{-1} (NEN)) in the same buffer

for 60 min at room temperature. After incubation, the sections were washed twice for 10 min at 4°C in fresh buffer. Non-specific binding was determined by use of 15 μM nimodipine (Sigma) under the same experimental conditions.

[^{3}H]Rolipram binding. Autoradiographic distribution of calcium/calmodulin-independent cyclic-AMP PDE inhibitor binding sites was detected using [^{3}H]rolipram according to the method of Kaulen et al (1989) with minor modifications (Araki et al 1992). Brain sections were incubated for 60 min at 0°C with 5 nM [^{3}H]rolipram (specific activity 60 Ci mmol^{-1}; Amersham) in 150 mM phosphate buffer (pH 7·4) containing 2 mM $MgCl_2$ and 100 μM dithiothreitol. After incubation the sections were washed twice with fresh buffer for 30 s at 0°C and briefly rinsed in ice-cold distilled water. Non-specific binding was determined using 1 μM rolipram (Meiji Seika Co. Ltd, Yokohama, Japan) under the same experimental conditions.

Data analysis

The sections were quickly dried under a cold air stream and were exposed to Hyperfilm-^{3}H (Amersham) for 4–6 weeks in X-ray cassettes with a set of [^{3}H]microscales (Amersham). The optical density of the brain areas was measured with a computer-controlled image analyser, as described previously (Araki et al 1995). Binding assays were performed in duplicate under subdued lighting. Values were expressed as means ± s.d. Statistical significance was determined by analysis of variance then Dunnett's multiple comparison test.

Results

Regional age-related alterations of [^{3}H]nimodipine and [^{3}H]rolipram binding are summarized in Tables 1 and 2. Representative autoradiograms of this binding are shown in Fig. 1.

Receptor autoradiography

[^{3}H]Nimodipine binding. The localization of [^{3}H]nimodipine binding was relatively heterogeneous throughout the brain. In adult (6-month-old) rats the highest [^{3}H]nimodipine binding was evident in the hippocampus, followed by the neocortex, thalamus, cerebellum and striatum. Other regions showed relatively low [^{3}H]nimodipine binding. In immature (3-week-old) rats a significant increase in [^{3}H]nimodipine binding was observed in the striatum and hippocampal CA3 sector and thalamus, compared with those regions in the adult animals. Other regions showed no significant change in [^{3}H]nimodipine binding. [^{3}H]nimodipine binding, on the other hand, was not significantly different in the brains of 12- and 18-month-old rats. In 24-month-old rats, however, a significant increase in [^{3}H]nimodipine binding was found in the striatum and hippocampal CA3 sector. Other regions showed no significant alteration in [^{3}H]nimodipine binding.

[^{3}H]Rolipram binding. In adult rats [^{3}H]rolipram binding was strikingly heterogeneous and was greatest in the hippocampus, neocortex, striatum and thalamus. Other regions had a low grain density of [^{3}H]rolipram binding. In immature rats the grain density of [^{3}H]rolipram binding was quite similar to that of the adult animals, although a significant increase in [^{3}H]rolipram binding was observed in the thalamus, substantia nigra and cerebellum of the immature rats, compared with those regions in the adult animals. Other regions showed no obvious change in [^{3}H]rolipram binding. No significant change in [^{3}H]rolipram binding was, on the other hand, seen in most brain areas in 12-, 18- and 24-month-old rats. A transient increase in [^{3}H]rolipram binding was observed only in the hippocampal CA1 sector of 12-month-old rats.

Discussion

Ageing is associated with striking changes in neurotransmission which might underlie age-dependent deficits in cognitive function and psychomotor performance (Bartus et al 1982; Joseph et al 1983). These deficits might, at least in part, be correlated with abnormal calcium metabolism. For example, calcium-dependent acetylcholine release is reduced in the brain of aged mice (Gibson & Peterson 1981) and perturbations in normal calcium metabolism have been correlated with learning and memory deficits in aged rabbits (Deyo et al 1989). Several studies have, furthermore, reported that brain ageing is accompanied by a reduction in calcium uptake (Gibson & Peterson 1987; Martinez et al 1987). Thus age-related changes in calcium metabolism might affect neuronal functions such as neurotransmitter release and neurotransmission.

In this study, no significant increase in [^{3}H]nimodipine binding was observed in any brain areas of 12- and 18-month-

Table 1. Age-associated changes in [^{3}H]nimodipine-binding in the rat brain.

Region	Age				
	3 weeks	6 months	12 months	18 months	24 months
Frontal cortex	35 ± 6	25 ± 11	24 ± 6	25 ± 10	37 ± 6
Parietal cortex	32 ± 6	30 ± 15	31 ± 7	28 ± 17	35 ± 7
Striatum	41 ± 8*	21 ± 10	25 ± 5	24 ± 10	43 ± 9*
Hippocampus					
CA1 sector	32 ± 6	31 ± 10	35 ± 10	24 ± 14	42 ± 10
CA3 sector	60 ± 10*	40 ± 9	47 ± 6	42 ± 14	63 ± 7*
Dentate gyrus	67 ± 10	75 ± 10	85 ± 11	72 ± 17	88 ± 9
Thalamus	66 ± 13*	30 ± 10	39 ± 6	33 ± 14	46 ± 10
Substantia nigra	32 ± 18	14 ± 12	17 ± 12	14 ± 14	27 ± 10
Cerebellum	37 ± 14	24 ± 5	21 ± 7	23 ± 9	22 ± 10

Optical density was converted to fmol (mg tissue)$^{-1}$ by use of [^{3}H]microscales. Values are expressed as means ± s.d. *$P < 0.01$ compared with 6-month-old group (Dunnett's multiple range test; n = 5–7).

Table 2. Age-associated changes in [^{3}H]rolipram-binding in the rat brain.

Region	Age				
	3 weeks	6 months	12 months	18 months	24 months
Frontal cortex	112 ± 20	102 ± 19	119 ± 16	91 ± 15	91 ± 15
Parietal cortex	100 ± 17	85 ± 14	106 ± 20	97 ± 17	90 ± 14
Striatum	73 ± 13	74 ± 81	82 ± 12	69 ± 13	77 ± 12
Hippocampus					
CA1 sector	104 ± 22	127 ± 15	156 ± 19*	148 ± 19	142 ± 20
CA3 sector	123 ± 23	123 ± 12	145 ± 32	129 ± 20	123 ± 16
Dentate gyrus	95 ± 14	89 ± 18	95 ± 18	85 ± 5	86 ± 15
Thalamus	138 ± 22†	87 ± 12	95 ± 16	82 ± 13	87 ± 18
Substantia nigra	64 ± 16†	43 ± 9	45 ± 8	41 ± 5	44 ± 8
Cerebellum	58 ± 12†	45 ± 7	36 ± 4	39 ± 3	37 ± 5

Optical density was converted to fmol (mg tissue)$^{-1}$ by use of [^{3}H]microscales. Values are expressed as means ± s.d. *$P < 0.05$, †$P < 0.01$ compared with 6-month-old group (Dunnett's multiple range test; n = 5–7).

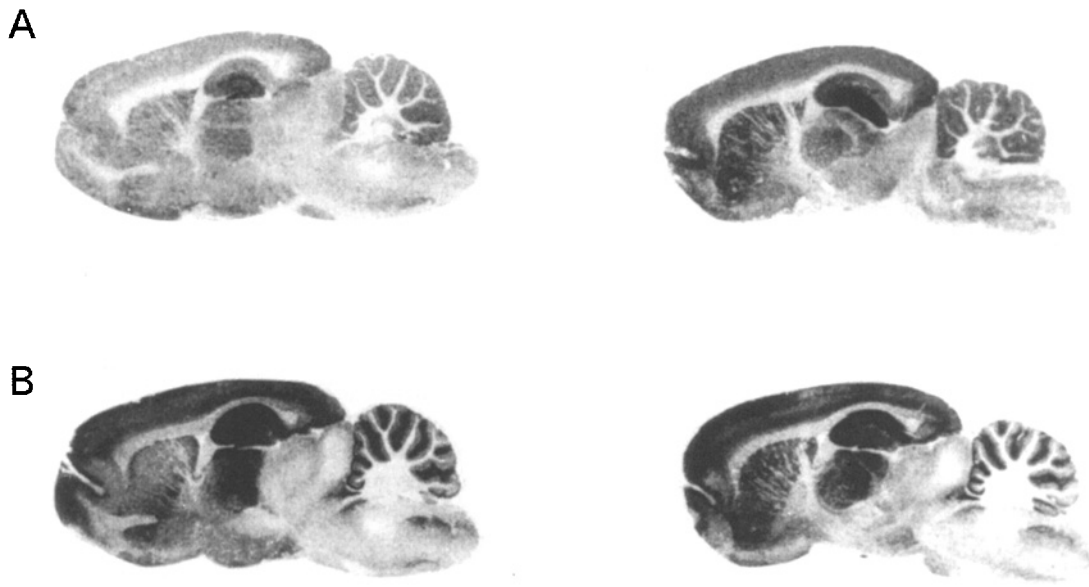

FIG. 1. Autoradiographic distribution of [^{3}H]nimodipine (A) and [^{3}H]rolipram (B) binding in the rat brain. Left: adult (6 months old) rat brain, right: aged (24 months old) rat brain. A significant increase in [^{3}H]nimodipine binding was noted in the striatum and hippocampal CA3 sector in aged rats, compared with those in adult animals (A). In contrast, no significant change in [^{3}H]rolipram binding was observed in any brain areas of aged rats (B).

old rats, as compared with those from adult animals. A significant increase in [^{3}H]nimodipine binding was, however, found in the striatum and hippocampal CA3 sector of 24-month-old rats. A previous study suggested that an age-dependent increase in [^{3}H]verapamil (a voltage-dependent calcium-channel blocker) binding was seen in the rat cortical membrane (Battaini et al 1985). Govoni et al (1985) reported, furthermore, that the number of [^{3}H]nitrendipine (a voltage-dependent calcium channel blocker) binding sites was slightly increased in the cortical membranes of 24-month-old rats. In agreement with our findings Navaratnam & Khatter (1991) have, interestingly, also observed an age-related increase in [^{3}H]nitrendipine binding in the sarcolemmal membrane. From these observations, the present study seems to suggest that calcium channels are gradually affected in ageing processes and this might lead to neurological deficits.

Several neurotransmitters are well known to stimulate the formation of cyclic-AMP by activation of adenylate cyclase (Phillis 1977). Cyclic-AMP plays a key role in expression or activation of ion channels and acetylcholine receptors (Artalejo et al 1990; Ifune & Steinbach 1990). The cyclic-AMP cascade, including adenylate cyclase, is involved in learning, short-term memory and synaptic plasticity (Buxbaum & Dudai 1989; Zhong et al 1992) and is, therefore, implicated in ageing processes and cognitive function. Cellular cyclic nucleotide phosphodiesterases are also known to play a role in the metabolism of cyclic-AMP by determining the response intensity and duration of neurons to various neurotransmitters acting via adenylate cyclase (Kaulen et al 1989).

Rolipram is an antidepressant with calcium/calmodulin-independent specific cyclic-AMP type-IV PDE inhibitory properties, leading to increased brain cyclic-AMP levels; the

binding sites have a unique distribution showing only limited overlap with general cyclic-AMP and phosphatidylinositol-related markers (Kariya & Dage 1988; Kaulen et al 1989). These observations, therefore, imply a function for the calcium/calmodulin-independent cyclic-AMP PDE in cognitive function and ageing processes. The present study, however, showed no significant changes in [^{3}H]rolipram binding in most brain areas during ageing. These findings suggest the possibility that calcium/calmodulin-independent cyclic-AMP PDE might not play a key role in ageing processes. It is, however, now generally accepted that there are four type-IV cyclic-AMP PDE isoform families, denoted IV_A, IV_B, IV_C and IV_D (Lobban et al 1994; McPhee et al 1995), although the precise functional role of these enzymes is not fully understood. It is, therefore, necessary to investigate the regional pattern of each subtype of type-IV cyclic-AMP PDE for further understanding of age-related changes in the brain, using receptor autoradiographic and biochemical techniques.

In this study, a significant increase in [^{3}H]nimodipine binding was observed in the striatum, hippocampal CA3 sector and thalamus of 3-week-old rats as compared with those of 6-month-old rats. On the other hand, [^{3}H]rolipram binding also increased significantly in the thalamus, substantia nigra and cerebellum of 3-week-old animals. Several recent studies have demonstrated that the density of calcium channels changes during development. In isolated rat hippocampal cells the peak calcium current increases from postnatal day 12 to postnatal day 28 (Thompson & Wong 1991). In isolated rat cortical neurons, furthermore, the mean whole-cell calcium current increases with age up to approximately 3 weeks postnatal and then levels off, with a decline in adult animals (Lorenzon & Foehring 1995). Thus the present study also seems to suggest that voltage-dependent L-type calcium channels change in the brain during development. Akaike et al (1993) recently reported that rolipram can contribute to the neuronal development of PC 12 cells by increasing intracellular cyclic-AMP, which has an important role in the development of voltage-dependent calcium channels. They suggest, furthermore, that rolipram can behave like a neurotrophic factor in cultured PC12 cells. This finding is of interest in relation to developmental relationships between the voltage-dependent calcium channels and calcium/calmodulin-independent cyclic-AMP PDE. In this study, however, we have no ready precise explanation of a significant increase in [^{3}H]rolipram binding in the thalamus, substantia nigra and cerebellum of 3-week-old rats. Further studies are, therefore, needed to investigate the detailed mechanism of our findings, although the study suggests that calcium/calmodulin-independent cyclic-AMP PDE and voltage-dependent L-type calcium channels might play roles in developmental processes.

In conclusion, this study provides evidence that voltage-dependent L-type calcium channels are more susceptible to ageing processes than calcium/calmodulin-independent adenosine monophosphate-selective phosphodiesterase in rat brains. Furthermore, our results suggest that voltage-dependent L-type calcium channels and calcium/calmodulin-independent adenosine monophosphate-selective phosphodiesterase might play roles in developmental processes. These findings might help further elucidation of the relationship between age-related neurological deficits and behavioural pharmacology including cognitive function.

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