A REVIEW OF AGE-RELATED CHANGES IN CEREBELLAR β -ADRENERGIC FUNCTION AND ASSOCIATED MOTOR LEARNING

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ABSTRACT

The present review provides an overview of age-related changes in cerebellar β -adrenergic function, associated motor learning, causal agents and possible treatments. Norepinephrine acts as a neuromodulator of Purkinje cell activity. With aging, however, the ability of norepinephrine to modulate Purkinje cell activity and specifically GABAergic inhibition of Purkinje cell activity is decreased. This age-associated deficit in cerebellar noradrenergic function correlates with deficits in acquisition of a motor learning task. Aged rats are delayed in acquiring a motor learning task that requires rats to adjust footfalls in order to cross a runway. The degree of deficit in cerebellar β -adrenergic activity correlated positively with the degree of impairment in task acquisition. One possible causal agent for the β -adrenergic deficit is free radical damage. Hyperoxia, which may generate free radical damage, induces cerebellar β -adrenergic deficits in young rats but diet restriction and treatment with antioxidants can delay or reverse age-related deficits in cerebellar β -adrenergic function in old rats.

KEY WORDS

Norepinephrine, Aging, Free Radicals, Antioxidants, Cerebellum, Rat

INTRODUCTION

The purpose of the present review is to focus on a series of experiments that studied age-related changes in cerebellar β -adrenergic function from the cellular level to the behavioral level. This systematic approach can be a valuable research tool because the behavioral manifestations of aging can be studied as well as the physiological basis for the behavioral changes. The combined behavioral and physiological approach may expedite development of ameliorative treatments. Thus, this review will concentrate on age-related changes in cerebellar β -adrenergic receptor function, age-related changes in an associated motor learning paradigm, possible causal mechanisms and ameliorative treatments.

The cerebellar β -adrenergic system is well suited for studies of aging because the system has been examined both at a cellular level and a behavioral level. A brief overview of cerebellar β -adrenergic function and associated behavior will aid in understanding age-related changes. The cerebellum is one of many neural areas that receives noradrenergic projections from the locus ceruleus. Noradrenergic fibers have been found in both cerebellar cortex (1, 2, 3, 4) and in the cerebellar deep nuclei (5, 6, 7). One function of norepinephrine (NE) in the cerebellum is to modulate Purkinje cell activity. For example, evoked inhibition of single-unit activity was recorded extracellularly from Purkinje cells both before and after micro-iontophoresis of NE. Norepinephrine enhanced the afferent evoked inhibition (8). Similarly, another study demonstrated that NE also augmented inhibition of Purkinje cell spontaneous activity produced by iontophoretic pulses of GABA (9). The level of NE that augmented GABAergic inhibition did not produce changes in the spontaneous firing rate when applied alone. Thus NE acts as a neuromodulator in the cerebellum by post-synaptically facilitating stellate and/or basket cell induced GABA inhibition of Purkinje cell firing. This facilitation of GABAergic inhibition of Purkinje cell firing is specific to the GABA_A receptor but not the GABA_B receptor (10, 11).

The NE-induced enhancement of GABAergic inhibition has been demonstrated specific to the β_1 -adrenergic receptor. The selective β_1 -receptor blocker practolol antagonized NE-induced enhancement of GABAergic inhibition of Purkinje cell firing in contrast to the β_2 -receptor blocker zinterol (12). In addition to being β_1 -receptor specific, it is known that noradrenergic modulation of GABAergic inhibition involves a second messenger signal transduction cascade that includes adenylyl cyclase activation and increased intracellular production of cAMP (13). Thus, NE, through activation of the β_1 -receptor signal transduction cascade, can enhance GABA_A-dependent inhibition of Purkinje cell firing. This amplification of cerebellar GABAergic responses may aid in acquisition of cerebellar dependent behaviors.

In fact, several studies have suggested that norepinephrine may enhance acquisition of tasks that are cerebellar dependent such as classical conditioning of the eye blink reflex in rabbits and adaptation of the

vestibular ocular reflex (for reviews of cerebellar dependent learning see 14 & 15). Propranolol (a β -adrenergic antagonist) delayed acquisition of classical eye blink conditioning relative to controls (16) and sotalolol (a β -adrenergic antagonist) reduced adaptation of the vestibular ocular reflex (17). Another learning paradigm that NE regulates is a runway paradigm in which rats must learn to adjust their gate in order to successfully navigate the runway. A study that looked at noradrenergic involvement in the runway task found that the degree of cerebellar noradrenergic deafferentation correlated with the degree of impairment in acquisition of the runway task but did not correlate with post-deafferentation motor performance (18). In addition, the β -adrenergic antagonist propranolol but not the α_1 -adrenergic antagonist prazosin or the α_2 -adrenergic antagonist yohimbine delayed acquisition of the runway task (19). Thus, the β -adrenergic system appears to be involved in acquisition of cerebellar based motor learning. β -adrenergic antagonists delay acquisition but do not prevent acquisition. One possible mechanism of NE involvement in acquisition of cerebellar based tasks may be modulation of GABAergic inhibition of Purkinje cell activity.

DISCUSSION

Aging and Cerebellar β -Adrenergic Function

Cerebellar β -adrenergic function is sensitive to detrimental effects associated with aging and this has been studied by examining 1.) receptor function, 2.) receptor subtype involvement, and 3.) signal transduction changes. Aged (18-20 months) and young (3 months) rats differed in the ability of NE to modulate Purkinje cell activity (20). Norepinephrine modulation of Purkinje cell evoked and spontaneous activity was compared between age groups. In young rats NE produced a greater decrease in spontaneous activity than in evoked activity. In contrast, for old rats NE produced a greater decrease in evoked activity. In addition, higher doses of NE were required in aged rats to produce an equivalent level of inhibition of spontaneous activity as recorded in young rats with lower doses. Similar differences were found for evoked activity. Norepinephrine increased parallel fiber evoked inhibition of Purkinje cell firing in young rats but did so to a lesser effect in aged rats. In a related study, NE modulation of complex spike discharge was assessed in Purkinje cells from young and old rats (21). Complex spikes were elicited by electrical stimulation of the inferior olive. In young rats (3-6 months) NE inhibited spontaneous activity but increased the likelihood of observing full sized action potentials in complex spike trains. The ability of NE to inhibit spontaneous activity and enhance complex spike trains was decreased in aged rats. Thus, aging is associated with a decrease in cerebellar NE modulation of both evoked and spontaneous Purkinje cell activity. In Figure 1, examples of the ability of the β -adrenergic agonist isoproterenol (ISO) to modulate GABAergic inhibition of Purkinje cell firing are shown from a young and an old rat.

Figure 1. The β -adrenergic agonist isoproterenol (ISO) enhanced GABAergic inhibition in a Purkinje cell recorded from a young (3 months old) but not from an old (24 months old) rat. 1.) A Purkinje cell was isolated in a young rat. A.) GABA was applied iontophoretically to establish a level of inhibition around 40%. B.) ISO was continuously applied and GABA was reapplied. The GABAergic inhibition was enhanced. C.) After ISO application, the level of GABAergic inhibition returned to baseline levels. 2.) A Purkinje cell was isolated in an old rat. A.) GABA was applied iontophoretically to establish a level of inhibition around 50%. B.) ISO was continuously applied and GABA was reapplied. The GABAergic inhibition was decreased. C.) After ISO application, the level of GABAergic inhibition returned to baseline levels. The response shown for the Purkinje cell from the old rat represents a deficit in β -adrenergic function.

It is possible that age-related changes in noradrenergic function are generalized to all noradrenergic receptors but it is also possible that only specific receptor subtypes are affected by aging. Studies have been conducted to specifically address this issue. Changes in Purkinje cell noradrenergic receptor subtype sensitivity were measured by comparing the ability of an α_1 -adrenergic agonist (phenylephrine), an α_2 -adrenergic agonist (clonidine) and the β -adrenergic agonist ISO to depress spontaneous Purkinje cell activity between young (3 months) and old rats (18 months & 26-28 months) (22). For both 18 and 26-28 month old rats, inhibition of spontaneous Purkinje cell activity by ISO was decreased relative to young rats. However, no subsensitivity to the α_1 and α_2 agonists were found for the aged rats. While this suggests that the cerebellar adenylyl cyclase-linked β -adrenergic system may be particularly sensitive to age-associated changes, it does not clarify which β -adrenergic receptor subtype is affected by aging. To address this point, the ability of β_1 -adrenergic receptor agonist dobutamine and the β_2 -adrenergic receptor agonist zinterol to decrease spontaneous Purkinje cell activity was tested in young (3 months) and old (18 & 26 months) rats (11). For 18 month old and 26 month old rats, dobutamine inhibition of spontaneous Purkinje cell activity was decreased compared to young rats but no age-related difference in sensitivity to zinterol was found establishing that aging produces a selective deficit in Purkinje cell β_1 -adrenergic receptor function.

The β_1 -adrenergic receptor is a G_s protein-linked receptor that activates adenylyl cyclase and increases intracellular cyclic AMP levels (13). The increased cAMP activates protein kinase A (PKA) which may ultimately potentiate Cl^- currents of the GABA_A receptor (23). It is possible that age-associated deficits in β -adrenergic receptor function may occur at any point or multiple points along the signal transduction cascade. Two studies examined age-related changes at different points in the signal transduction cascade. The first study looked at the ability of locally applied NE and cyclic adenosine-mono-phosphate (N6cAMP) to decrease Purkinje cell activity in young (4 months) and old (15-22 months) rats (24). In addition, sensitivity to GABA was also measured. Purkinje cells from young rats were more sensitive to NE and N6cAMP than Purkinje cells from old rats but no difference in sensitivity to GABA was found. These results suggest that the decreased sensitivity to NE may occur at or beyond the point of cAMP generation.

The second study focusing on age-related deficits in the cerebellar β -adrenergic signal transduction cascade examined possible changes in adenylyl cyclase activity and cAMP activity (Fig. 2) (25). In young rats the β -adrenergic agonist ISO can enhance GABAergic inhibition at doses below levels of ISO that alter spontaneous Purkinje cell activity. The deficit in the ability of ISO to increase GABAergic inhibition in aged rats could occur anywhere along the β -adrenergic signal transduction

Figure 2. The β_1 -adrenergic receptor is a G_s protein-linked receptor that activates adenylyl cyclase and increases intracellular cyclic AMP (cAMP) levels that in turn activate protein kinase A (PKA) which may ultimately potentiate Cl^- currents of the GABA_A receptor. In aged rats, forskolin was used to directly activate adenylyl cyclase and Sp-cAMPS was used to directly activate PKA as an attempt to bypass the age-related deficit in β_1 -adrenergic receptor function.

cascade. To test this forskolin, which stimulates adenylyl cyclase activity (26), was used to activate the β -adrenergic signal transduction cascade at the point of adenylyl cyclase activation in young (3 months) and old rats (18-21 months). If the age-related deficit in β -adrenergic function occurs before adenylyl cyclase activation, then forskolin should enhance GABAergic inhibition in both young and old rats. Forskolin, however, only enhanced GABAergic inhibition in young rats suggesting that a deficit in the β -adrenergic signal transduction cascade occurred at or beyond activation of adenylyl cyclase while also not ruling out a deficit before adenylyl cyclase activation. The same experiment also used an analog of cAMP, adenosine-3',5'-cyclic monophosphothioate Sp-isomer (Sp-cAMPS), to directly activate PKA in young and old rats. Again, if the age-associated deficit occurs before PKA activation then use of Sp-cAMPS should bypass the age-related deficit. The Sp-cAMPS compound enhanced GABAergic inhibition in both young and old rats but higher doses were required in the old rats. This result suggests that an age-related deficit in cerebellar β -adrenergic function occurs at the point of PKA activation. Thus both the Marwaha et al. study (24) and the Gould and Bickford study (25) suggest that aging may affect the cerebellar β -adrenergic signal transduction cascade at the point of cAMP activation of PKA.

It should be pointed out that age-related changes in cerebellar β -adrenergic function are not limited to the signal transduction cascade. Selective changes in β -adrenergic receptor binding do occur with age. With age, binding of the non-selective β -adrenergic antagonist pindolol decreased in the cerebellum, thalamus and brainstem but no changes occurred in the hippocampus or cortex of rats (27). Another study looked specifically at cerebellar β -adrenergic receptor subtypes in aged rats and found that β_2 -adrenergic receptor density decreased with age but β_1 -adrenergic receptor density increased with age (28). This result is not surprising because subsensitivity for the β_1 receptor subtype has

been reported but not for the β_2 receptor subtype (11). Thus, upregulation of the β_1 receptor subtype may be a compensatory reaction to the decreased sensitivity of the β_1 receptor due to deficits in signal transduction.

Aging and Cerebellar β -Adrenergic Associated Motor Learning

To this point the work reviewed details an age-related deficit in cerebellar β -adrenergic function with the point of cAMP activation of PKA in the signal transduction cascade being particularly sensitive to the effects of aging but three important issues still need to be addressed: 1.) the behavioral manifestation of the biological deficit, 2.) casual factors, and 3.) possible treatments. If the cerebellar β -adrenergic system is involved in acquisition of cerebellar based tasks, then age-associated deficits in cerebellar β -adrenergic function should be correlated with age-associated deficits in cerebellar-based learning. To assess the relationship between β -adrenergic function and motor learning, a study compared acquisition of the runway paradigm between aged (20 months), and young (3-6 months) rats that either were controls or were depleted of central stores of NE via 6-hydroxydopamine-lesions (6OHDA) (29). All groups acquired the task but the young control group had a steep acquisition curve indicating rapid acquisition of the task whereas the old and young 6OHDA lesioned groups had shallow acquisition curves indicating prolonged acquisition of the task. The average acquisition curves for the 6OHDA depleted young rats and old rats were not different but both were different from the average acquisition curve for the young control rats. This study also demonstrated that the impairment in task acquisition was dissociated from motor impairment. Rats were tested on a motor performance battery, and errors in motor performance did not correlate with motor learning deficits. Results from the Bickford et al. study (29) suggest that age-related changes in noradrenergic function are related to age-related changes in motor learning.

The finding that age-related changes in noradrenergic function are related to age-related changes in motor learning was strengthened by results from further studies of aging that directly examined both motor learning and β -adrenergic function in the same animals. Both young (3 months) and old (20 months), male and female rats were trained on acquisition of the runway paradigm (30, 31). After behavioral training and testing, cerebellar β -adrenergic function was assessed through use of pharmacological and electrophysiological techniques. Rats were anesthetized and the ability of the β -adrenergic agonist ISO to modulate GABAergic inhibition of Purkinje cell firing was assessed. Aged rats had deficits in both acquisition of the motor learning task and in β -adrenergic function. In addition, acquisition rates were correlated with the degree of impairment in β -adrenergic function. Thus, there is strong evidence that age-related deficits in cerebellar β -adrenergic function contribute to age-related impairments in motor learning. The mecha-

nisms responsible for producing the age-related impairments, however, remain uncertain.

Aging, Free Radicals and Cerebellar β -Adrenergic Function

One theory of aging that has been receiving increasing attention is the free radical theory. Free radicals are a byproduct of cellular oxidative metabolism and are very reactive species that can generate cellular damage. Thus, the accumulation of free radicals during an organism's life span may lead to age-associated deterioration of function (32). If the free radical theory of aging is correct then variations in the rate of accumulation of free radical damage should produce variations in the rate of aging. This theory has been tested by manipulating levels of free radicals in studies that increased free radical damage via exposure to normobaric hyperoxia and studies which decreased free radical levels via pharmacological agents. Rats exposed to hyperoxia had increased levels of protein carbonyl (a measure of protein peroxidation) and increased levels of reactive oxygen species in brain (33, 34). Thus it may be expected that hyperoxia would produce deficits in cerebellar β -adrenergic function similar to those associated with aging. β -adrenergic function was assessed in young (3-4 months) rats exposed to 48 hours of normobaric hyperoxia and control young rats. Indeed, the young hyperoxia-exposed rats had deficits in cerebellar β -adrenergic function similar to deficits seen in old rats (35). In addition, the same study looked at β -adrenergic function in a line of rats that were bred for resistance to the toxic effects of normobaric hyperoxia. Young hyperoxia-resistant rats did not have deficits in cerebellar β -adrenergic receptor function after a 48 hour exposure to normobaric hyperoxia. In addition, cerebellar β -adrenergic function was compared between 18 month old hyperoxia-resistant rats and 18 month old control rats. These rats were not exposed to normobaric hyperoxia but instead were tested for differences in the effects of aging on β -adrenergic function. Isoproterenol modulated GABAergic inhibition in more Purkinje cells from old hyperoxia-resistant rats than from the age-matched control rats. In fact, the old hyperoxia-resistant rats had a level of β -adrenergic function similar to young rats. This study suggests that deficits associated with aging and hyperoxia may share a common mechanism; perhaps free radical damage.

If free radical damage does contribute to age-related deficits then lowering free radical levels should reduce deficits associated with aging. Multiple methods may successfully lower free radical levels and some methods that have been tested include the use of spin trapping agents, antioxidants, and diet restriction. N-tert-butyl- α -phenyl-nitrone (PBN) is a spin trapping agent that can scavenge free radicals. Twenty-one to twenty-two month old rats were treated with PBN for two weeks after which time cerebellar β -adrenergic function was compared between PBN-treated rats and age-matched control rats

(36). For the PBN-treated rats, ISO produced modulation of GABAergic inhibition in more Purkinje cells compared to age-matched controls. In fact, β -adrenergic function in the PBN-treated rats resembled β -adrenergic function in young rats. These results are similar to other work that has demonstrated ameliorative effects of PBN on age-related deficits (37, 38). These findings not only suggest that PBN may ameliorate some age-related deficits but also provides further support for the free radical theory of aging.

Other antioxidant agents should also ameliorate age-related deficit in cerebellar β -adrenergic function. To test this, 18 - 20 month old rats were treated with either the spin-trapping agent MDL 101.002 (MDL) or a diet supplemented with vitamin E for two weeks before assessing cerebellar β -adrenergic function (39). The ability of ISO to modulate GABAergic inhibition of Purkinje cell spontaneous activity in both the MDL-treated and vitamin E-treated old rats was similar to that reported for young rats but was superior to that recorded from age-matched control rats. This demonstrates that the ameliorative effects reported for PBN also generalized to non-nitron family spin trapping agents such as MDL and antioxidants such as vitamin E and also suggests that the ameliorative actions of these compounds for age-related changes in cerebellar β -adrenergic function may be related to reduction of free radical levels. Treatments with PBN, MDL and/or vitamin E may be effective treatments for some of the deleterious effects associated with aging but other treatments may also be possible.

Diet restriction is one manipulation that has consistently countered some of the deleterious effects of aging (40, 41, 42, 43, 44, 45, 46, 47, 48). While it is not completely clear how diet restriction retards some of the damaging effects associated with aging, increasing evidence suggests that diet restriction may reduce free radical damage (32, 50, 51). In support, diet restriction has been shown to reduce lipid peroxidation and decrease generation of mitochondrial superoxide and hydrogen peroxide (44, 49). Thus, reduction of caloric intake through diet restriction may decrease accumulation of free radical damage.

If diet restriction is indeed reducing age-associated free radical damage, then diet restriction should also produce ameliorative effect on cerebellar β -adrenergic function in aged rats similar to those produced by antioxidants. To test if diet restriction could prevent or delay age-related deficits in cerebellar β -adrenergic function, rats were either fed ad lib or with a diet consisting of a 40% reduction in calories starting at 4 months of age and age-related changes in motor learning and β -adrenergic function were examined. Age-related changes in acquisition of the motor learning runway paradigm were assessed at 14 months for one experiment and 22 months of age for a second experiment. After behavioral testing, cerebellar β -adrenergic function was tested in each animal by measuring the ability of the β -adrenergic agonist ISO to modulate GABAergic inhibition of Purkinje cell firing (52).

Both 14 month old and 22 month old diet restricted rats reached a criterion of performing 10 successful crosses in 10 minutes sooner than age-matched ad lib fed rats. In addition, the 22 month old diet restricted rats had superior acquisition times compared to age-matched ad lib fed rats. Rats were also tested for differences in strength and coordination. No consistent differences were seen in motor performance between ad lib fed and diet restricted rats suggesting that differences in motor learning were not primarily do to differences in strength and coordination.

Cerebellar β -adrenergic function was examined at 14, 22 and 24 months in diet restricted and ad lib fed rats. For all groups of diet restricted rats, ISO modulation of GABAergic inhibition of Purkinje cell firing was greater than that recorded in age-matched ad lib fed rats. However, no difference in the number and affinity of cerebellar β -adrenergic receptors was found between ad lib and diet restricted rats. However, results indicated that the β_2 -receptors most likely were the only receptor subtype to be detected. Thus it is not entirely surprising that no difference was found since the age-related change in β -adrenergic function is specific to the β_1 -receptor (11). The Gould et al. study on diet restriction (52) does demonstrate that diet restriction can reduce or delay development of age-related deficits in both cerebellar β -adrenergic receptor function and in associated motor learning.

CONCLUSION

The present review has focused on age-related changes in cerebellar β -adrenergic function and associated behavior. Strong evidence has been provided demonstrating that with age cerebellar β -adrenergic function decreases and this decrease is associated with deficits in motor learning. In addition, this work has provided a clue as to a possible mechanism of these age-related deficits and that mechanism could be free radical damage. Understanding the mechanism responsible for age-related deficits may help in developing treatments for both age-related deficits and age-associated diseases. Thus, the research approach of studying aging at both the behavioral and related neuronal levels has provided a useful means for further understanding mechanisms of aging.

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