AGE-RELATED ELECTROPHYSIOLOGICAL CHANGES IN CEREBELLAR NORADRENERGIC RECEPTORS

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ABSTRACT

Noradrenergic transmission in the central nervous system declines early in the aging process. This decline can be demonstrated as subsensitivity to the depressant effects of norepinephrine on cerebellar Purkinje neurons of aged rats. In young rats alpha1, alpha2, and beta adrenergic receptors are present and functional in the cerebellar cortex. The purpose of this study was to determine which of these receptor subtypes alter their response with age. Inhibition of the spontaneous activity of Purkinje neurons by selective noradrenergic agonists was compared in young (3-month-old) and aged (18- and 26-28-month-old) Fischer 344 rats. These agonists were applied to Purkinje neurons by pressure microejection from multi-barreled micropipettes and the change in neuronal action potential discharge rate was recorded. Purkinje cells of both groups of aged rats were significantly less sensitive to locally-applied Isoproterenol, a beta-adrenergic agonist, than Purkinje cells of young rats. Subsensitivity to the alpha agonist phenylephrine and the alpha2 agonist clonidine was not observed in the aged rats. These results suggest an age-related functional decline in an adenylate cyclase-linked beta receptor system with no concomitant functional change in receptor systems linked to other second messengers.

INTRODUCTION

The changes in gait, balance, and coordination that are hallmarks of advancing age may be associated with changes in neurotransmitter function in the central nervous system. Some specific neural pathways and neurotransmitters that are altered with senescence and contribute to dysfunction in motor performance have recently been identified (1-3); nevertheless, the mechanisms underlying age-related changes in specific neural systems remain poorly understood.

One brain region known to play a critical role in movement and balance regulation is the cerebellum (1, 2, 4, 7, 8). Lesions of the cerebellum result in a loss of balance, coordination, smooth execution of movement, and ability to learn new motor tasks (7-11). The noradrenergic projection from the nucleus locus coeruleus to the cerebellar cortex is thought to be an important modulator of fine motor control (2, 9, 12). Electrophysiological studies in anesthetized rats have shown that stimulation of the locus coeruleus, or local application of norepinephrine, results in an inhibition of Purkinje cells, the major output neurons of the cerebellar cortex (13-15). The locus coeruleus is capable of influencing not only the spontaneous activity of Purkinje cells, but also functions as an important modulator of the activity of other cerebellar neurons (16, 17); NE in the cerebellum can enhance the inhibitory actions of GABA (released by inhibitory interneurons) or the excitatory actions of glutamate (putatively released by the parallel fiber axons of granule cells).

Deficits in cerebellar noradrenergic neurotransmission have been observed in a number of studies. Purkinje neurons of aged rats are significantly less sensitive to locally-applied NE (18-20), to locus coeruleus activation (19), and to locally-applied phencyclidine (18), an indirect noradrenergic agonist (21). Age-related changes in the modulatory properties of NE have also been reported (22, 23). These changes in postsynaptic sensitivity occur as early as 18 months of age. Similar changes in response to GABA, the other major inhibitory transmitter of the cerebellum, are not observed (19).

The goal of this study was to identify the receptor subtype(s) responsible for the diminished postsynaptic sensitivity observed at cerebellar Purkinje neurons of aged rats. Recent evidence has demonstrated that alpha1, alpha2, and beta receptors are all functional in the cerebellar cortex, and that there is an inhibitory effect of noradrenergic agonists for all three receptors (24). Beta receptors, moreover, seem to play an important role in mediating the noradrenergic enhancement of GABA neurotransmission (25). Three age groups were chosen for the study: 3-month-old rats were selected as the youngest group because at this age the animals are sexually mature and past the stage of rapid brain growth. Eighteen-month-old rats were included as a senescent group because changes in noradrenergic function have been observed at this age (18-20, 22, 23, 26), and yet other senescent changes, such as a decrease in Purkinje cell spontaneous firing rate, are not seen (18, 28). Twenty-six-twenty-eight-month-old rats were used as the most elderly group based on the suggestion (27) that animals at their age of average...
longevity are comparable to human males 70-80 years of age, and also because the morphological markers of senescence are strikingly apparent in rats of this age (28-33).

RESULTS

Electrophysiological responses of cerebellar Purkinje neurons to locally-applied adrenergic agonists were evaluated in young (3-month-old), aged (18-month-old) and very aged (26-28-month-old) Fischer 344 rats. Each drug was tested in 9 young, 5 aged, and 4 very aged rats. Local application of phenylephrine, clonidine, or isoproterenol to Purkinje cells produced a dose-dependent inhibition of spontaneous cell firing rate, as described previously (24). These agonists and their doses were chosen on the basis of their previously demonstrated selectivity for alpha₁, alpha₂ and beta receptors (respectively) at cerebellar Purkinje neurons (24). For each neuron, several doses of an agonist were tested to determine a dose which produced a 40-60% decrease in Purkinje cell spontaneous activity; this approximate ED₅₀ dose was tested at least three times to ensure a consistent response.

Figure 1. Ratemeter records showing the responses of cerebellar Purkinje neurons of 3-month (A1 and B1), 18-month (A2), and 26-month (B2) Fischer 344 rats to local application of phenylephrine (PE). Several doses of the agonist were tested to determine a dose which produced an approximate 50% inhibition of Purkinje cell firing rate. A paired-pipette paradigm was used, in which the pipette in (A2) was the same as that used in (A1) and the pipette in (B2) was the same as that used in (B1). Purkinje cells of the aged rats responded to doses of PE that did not differ significantly from those that were effective in the young rats. Dose in this and subsequent ratemeter records is expressed in terms of pounds per square inch (p.s.i., noted above drug applications) x seconds (as indicated by the duration of the bars above the tracing). Numbers below the bars indicate the percent inhibitory response to drug applications, determined as described in Experimental Procedures. Calibrations: vertical—25 action potentials per second; horizontal—20 seconds.

Figure 1 is a ratemeter record from a typical experiment, showing the response of Purkinje neurons from 3-month-old (A1 and B1), 18-month-old (A2) and 26-28-month-old (B2) F344 rats to local, pressure-ejected applications of the alpha agonist, phenylephrine. Each vertical deflection of the ratemeter pen indicates the number of action potentials per second of the neuron being recorded. In order to eliminate variance due to differences in release of drug from different micropipettes, the same pipette was used to record from a 3-month-old rat and an aged rat in Figure 1A1 and 1A2; the same is true of 1B1 and 1B2. Figure 1A1 shows that a dose of 31 p.s.i. x 5 sec. (or 155 p.s.i. x sec) produced a 40-60% decrease in spontaneous discharge rate of the cell from a young rat. Figure 1A2 shows that a similar dose of the drug induced Purkinje cell inhibitions of a similar magnitude in an 18-month-old rat. Similar effects are seen in Figure 1B with a 26-month-old rat. The ratemeter records in Figure 2 show that, as with phenylephrine, there were no apparent age-related changes in sensitivity to the alpha agonist, clonidine; Purkinje cells of the 18-month-old and 26-28-month-old animals showed 40-60% responses to doses of clonidine that were equally effective as those in the 3-month-old animals.

The ratemeter records of Figure 3, however, illustrate that there was an age-related decrease in sensitivity to the beta agonist, isoproterenol; a dose of 15 p.s.i. x sec was effective in inducing a 40-60% inhibition of Purkinje cell spontaneous activity in a young rat (Fig. 3A1), whereas even very high doses of isoproterenol (1650 p.s.i. x sec., Figure 3A2) from the same micropipette failed to inhibit Purkinje cell activity in an 18-month-old rat. This decreased sensitivity to isoproterenol was also observed in the 26-28-month-old rats: the aged animals failed to respond to high doses of isoproterenol (Figure 3B2), whereas Purkinje cells of pipette-matched 3-month-old animals responded to low doses of the agonist (55 p.s.i. x sec., Figure 3B1).

A nonlinear regression curve-fitting program (see Experimental Procedures) was used to construct population dose-response curves based on the ED₅₀ values from the population of cells recorded for each drug and each age group. This analysis yielded the maximum percentage of cells responding to locally applied agonist and a population ED₅₀, which is the dose of drug at which half of the responsive neurons show a 50% decrease in firing rate. The Kolmogorov-Sminnro statistic was then used to distinguish differences in responses to the noradrenergic agonists between the young and aged populations.

Figure 4 shows cumulative dose-response curves from the three age groups for the number of Purkinje cells responding to phenylephrine. Comparison of the dose-response curves reveals no age-related change in response to phenylephrine; there was no evidence of change in either the population ED₅₀ (63.7 p.s.i. x sec., 95% Con-