Specific decrease of high-affinity agonist states of alpha$_2$-adrenoceptors in the aging mouse brain

Short Communication

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Summary. Using the specific binding of the full alpha$_2$-adrenergic agonist $^3$H-UK-14,304 the contribution of high-affinity agonist states to the total number of alpha$_2$-adrenoceptors as labeled by the specific binding of the antagonist $^3$H-yohimbine has been investigated in the brain of young and aged mice. In contrast to findings with human platelet membranes, in young mice all central alpha$_2$-adrenoceptors are present in a high-affinity agonist conformation. While the total number of alpha$_2$-adrenoceptors was not changed in the brain of aged animals, a specific decline of the high-affinity agonist sites by about 30% was observed. It is suggested that the specific decrease of high-affinity agonist sites of central alpha$_2$-adrenoceptors might represent one of the mechanisms leading to a general impairment of central noradrenergic neurotransmission with aging.

Keywords: Alpha$_2$-adrenoceptor, high-affinity agonist binding sites, aging.

Introduction

A variety of data indicate that pharmacological manipulations leading to increased noradrenergic activity in the CNS improve cognitive performance in animals and man and the reverse is the case for manipulations decreasing central noradrenergic activity (Zornetzer, 1985; Arnsten and Goldman-Rakic, 1987; Davis et al., 1988). Since noradrenergic neurotransmission is impaired in the course of normal aging in animals and man and is further substantially reduced in dementia of the Alzheimer's type (SDAT) with a pronounced loss of catecholaminergic cells of the locus coeruleus, it has been suggested that deficits of central noradrenergic neurotransmission might contribute to the impairment of cognitive functions in age-associated memory impairment (AAMI) and SDAT (Bondareff et al., 1982; Carlsson, 1987).
One possible strategy to substitute for the noradrenergic deficit is treatment with alpha2-adrenergic agonists like clonidine. While clonidine usually impairs mental performance when given to young animals or young man, recent data suggest that clonidine can improve cognitive functions in aged rodents as well as aged monkeys (Arnsten and Goldman-Rakic, 1985, 1987; Joly and Sanger, 1988). However, negative findings have also been reported (Bartus and Dean, 1988; Davis et al., 1988). The biochemical basis of the specific responsiveness of aged animals to clonidine is not known, especially since the loss of noradrenergic cells in the course of normal aging is rather small (Carlsson, 1987). Since age-related functional deficits of central alpha2-adrenoceptors could be an alternative explanation, we investigated whether normal aging alters the properties of central alpha2-adrenoceptors, using the specific binding of the antagonists 3H-yohimbine to label the total receptor population irrespective of the affinity state as well as the specific binding of the full agonists 3H-UK-14,304 [5-bromo-6(2-imidazoline-2-ylamino)-quinoxaline] which only labels the high-affinity agonist state of the alpha2-adrenoceptor (Schloss et al., 1987; Neubig et al., 1988).

**Materials and methods**

**Material**

Young (3 months) and aged (18 months) female NMRI mice were obtained from Interfauna (Tutlingen, Federal Republic of Germany). It should be noted that in respect to their average life span mice at the age of 18 months can be considered as aged but not as extremely old. The animals were killed by decapitation and the brains were quickly removed. The whole brains (except brain stem and cerebellum) were homogenized in 50 mmol/l Tris-HCl buffer pH 7.4 (containing 0.8 mmol/l EDTA) and centrifuged at 20,000 × g for 20 min. 3H-yohimbine (specific activity 88 Ci/mmol) and 3H-UK-14,304 (imidazolyl-4.5-3H) (specific activity 63 Ci/mmol) were purchased from New England Nuclear (Dreieich, Federal Republic of Germany). All other chemicals were obtained from commercial suppliers. Protein concentrations were determined according to the Lowry method (Lowry et al., 1951).

**Binding assays**

The supernatant was discarded and the pellet was resuspended in the same buffer, divided in two parts and centrifuged again. The final pellets were resuspended in the individual incubation buffers to give a final tissue concentration corresponding to about 10 mg original wet weight per ml. The following buffers (both pH 7.4) were used: 3H-yohimbine binding (50 mmol/l Tris-HCl, 0.8 mmol/l EDTA, 0.8 mmol/l ascorbic acid, 0.9% NaCl) and 3H-UK-14,304 (50 mmol/l Tris-HCl, 0.8 mmol/l EDTA, 0.8 mmol/l ascorbic acid, 0.3 mmol/l catechol, 0.3 mmol/l dithiothreitol, 10 mmol/l MgCl) (Schloss et al., 1987). Assays were performed in triplicate in a final volume of 300 μl which consisted of 200 μl membrane suspension, 50 μl radioligand and 50 μl competitor or blank. Incubations were performed at room temperature for 30 or 70 min for 3H-yohimbine or 3H-UK-14,304 binding, respectively, after which time binding of both radioligands was in equilibrium. Binding was terminated by rapid filtration through Whatmann GF-C filters under slight vacuum. Filters were washed 3 times (3H-yohimbine) or 4 times (3H-UK-14,304) with 5 ml ice cold buffer pH 7.4 (50 mmol/l Tris-HCl, 0.8 mmol/l EDTA).