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Research report

Age-related deficit in behavioural extinction is counteracted by long-term ethanol consumption: Correlation between 5-HIAA/5HT ratio in dorsal raphe nucleus and cognitive parameters

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Abstract

We investigated age-related changes in learning and memory performance and behavioural extinction in the water maze; and in endogenous levels of serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in the neocortex, hippocampus, thalamus and dorsal raphe nucleus of Wistar rats. Another aim was to assess the correlation between behavioural and biochemical parameters, which were measured in rodents of two different ages: 5 months (adults) and 16 months (middle-aged). The middle-aged subjects succeeded in learning the behavioural task, albeit with significantly worse performance when compared to adult animals. Aging also had significant main effects on memory and extinction. An age-dependent decrease in 5-HIAA levels was observed in both hippocampus and dorsal raphe nucleus (DRN). The decrease in DRN 5-HIAA was paralleled by a decrease in 5-HIAA/5-HT ratio in this brain area, which was significantly correlated to the animals' spatial memory performance and behavioural extinction. In addition, using middle-aged rats, a 2 × 2 factorial study was carried out to examine the effects of food restriction and chronic ethanol consumption on rat's performance in a spatial behavioural task and on central serotonergic parameters. None of these two treatments had a significant effect on the behavioural and biochemical parameters assessed, with the exception of extinction index, which was significantly affected by ethanol consumption. Long-term ethanol ameliorated the impairment in behavioural flexibility caused by aging. In conclusion, long-term ethanol consumption may have a role in protecting against age-related deficit in behavioural extinction. Moreover, the present results also indicate that DRN serotonergic system is involved in spatial memory and behavioural extinction.

Keywords: Aging; Caloric restriction; Chronic ethanol; Spatial memory; Behavioural extinction; 5-HT; 5-HIAA; Rats

1. Introduction

During normal aging, various cognitive processes, including learning and memory, are profoundly affected in both humans [65,77,22] and rodents [25,26,66,33]. De Luca et al. [18] showed that old subjects have decreased performance in tasks requiring cognitive flexibility, providing support for the hypothesis of a vulnerability of executive skills to normal aging. Accordingly, using an animal model, Topic et al. [79] showed that behavioural extinction is affected during aging. However, although there are some studies about extinction processes [34,43,44,64,67,78], the biological substrates involved in this behavioural phenomenon are still unknown. While the role played by the cholinergic system in age-related cognitive impairment has been extensively investigated [14,21,71], relatively few studies have examined whether the central serotonergic system

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has a role in the age-related impairments in spatial learning, memory and behavioural extinction. Some studies show an age-related emotional dysfunction associated to changes in central serotonergic parameters [47]. In addition, studies have yielded different answers regarding the involvement of the central serotoninergic system in spatial memory. Some authors, for instance, using different tools to induce depletion in brain serotonin, demonstrated that low serotonin levels had no effect on spatial memory performance [38,80], whereas other groups found that the serotonergic system is involved with spatial learning and memory [41,73]. There is a putative interaction between 5-HT and other neurotransmitters, e.g. acetylcholine, in learning and memory [16,74,84], and this interaction is probably disturbed during aging [47,63]. It has been shown that cognitive deficits related to aging might involve concomitant alterations of cholinergic, dopaminergic, noradrenergic and serotonergic systems in several brain regions such as the striatum, hippocampus or cortex [75]. However, so far, to our knowledge, the correlation between age-related alterations in region-specific serotoninergic parameters and cognitive deficits, such as in behavioural extinction, has not been investigated.

The hippocampal and neocortical formations play well-recognized roles in cognitive functions and are both known to be affected during aging [3]. Yet, the precise manner whereby these structures interact to process memory remains to be determined. It is clear that there are strong interconnections between the hippocampus and prefrontal cortex, in part mediated through thalamic nuclei [9,83,86]. Moreover, Mair et al. [40] showed that lesions in the anterior thalamus and parahippocampal area appear to have separate effects, which together disrupt hippocampus-dependent spatial memory. However, neurochemical systems underlying these connections are not well elucidated, and one possibly involved is the serotonergic. This is so because the dorsal raphe nucleus, which contains the largest concentration of serotonin neurons in the brainstem, provides the majority of serotonergic innervation to the forebrain [29]. In particular, thalamic nuclei, hippocampus and neocortex are innervated by serotonergic fibres from dorsal raphe nucleus [6,48]. A question to be clarified is whether these serotononergic pathways are, in fact, involved in the age-related cognitive dysfunction.

The molecular basis of the age-related neurochemical and cognitive changes is unknown, but many studies implicate reactive oxygen species and metabolic stress [24,42,53,70]. Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals [72].

There is evidence that caloric restriction (CR) decreases the rate of mammalian aging, and that it has many beneficial effects on the brain of rodents and possibly of humans [85]. Hemond and Jaffe [31] showed that caloric restriction-induced changes in calcium accumulation and membrane excitability may in part account for the protective effects of dietary restriction against deficits in synaptic plasticity and learning in aged animals. Thus, caloric restriction has been shown to delay or reduce the onset of most age-related diseases and to alter most physiological processes that change with age [23,45,72]. For instance, aged rats reared on a restricted diet display a substantial reduction in age-related memory defects [32,61].

Several external factors, such as chronic ethanol consumption, could be implicated in the age-related cell injury [56]. Chronic intake of ethanol is also known to impair cognitive function [1,15,46] and to induce free radical production, thus causing oxidative damage [81]. Free radicals have been hypothesized to contribute to the action of ethanol on the central nervous system, particularly in aged subjects [13]. The role of oxidative stress in alcohol-induced neurotoxicity is also supported by studies showing beneficial effects of antioxidant therapy during alcohol exposure [4]. Also in accordance with such hypothesis, a previous study from our group showed that, in adult rats, CR protected against chronic-ethanol-induced behavioural deficits, and that the mechanisms underlying this protection could involve prevention of oxidative stress [58]. Moreover, age-related cognitive deficits are also likely to bear relation to oxidative stress, as the brain seems to become increasingly vulnerable to such processes during aging [69].

With the aim of elucidating the age-related changes in both cognitive aspects and central serotonergic parameters and the effects, during aging, of chronic ethanol consumption associated or not to a caloric restricted diet, we decided to measure the following parameters: (i) animals' performance (spatial learning and memory and behavioural extinction) in the water maze spatial task and (ii) serotonin, 5-hidroxy-indol-acetic acid concentrations and 5-HIAA/5-HT ratio in neocortex, hippocampus, thalamus and dorsal raphe nucleus. The correlations between these biochemical and behavioural parameters were also assessed. In addition, the effects of the administration of a restricted diet and/or chronic ethanol were studied in old rats, to analyze possible effects of these treatments on both central serotonergic parameters and rat performance in a spatial behavioural task.

2. Material and methods

2.1. Animals and treatment

Thirty-two male Wistar rats aged 3 months in the beginning of the experiment were divided into four groups ($n = 8$ each), according to diet and administered fluid, as follows: (i) group Middle-aged, Standard, Water (MSW), in which animals received ad libitum commercial chow and tap water; (ii) group Middle-aged, Standard, Ethanol (MSE), in which animals received ad libitum commercial chow and 20% (v/v) ethanol solution, as the only source of fluid available; (iii) group Middle-aged, Restricted, Water (MRW), in which animals received 50% of the daily average amount of chow consumed by group MSW in the previous week, and ad libitum tap water; (iv) group Middle-aged, Restricted, Ethanol (MRE), in which animals received 50% of the daily average amount of chow consumed by group MSW in the previous week, and ad libitum 20% (v/v) ethanol solution as the only source of fluid available. Initial ethanol solution concentration was 5% (v/v) and was increased every 2 days by 5%, until reaching the final concentration of 20% (v/v), which was maintained for 12 months. Ethanol withdrawal was done by progressively decreasing concentration 5% every 2 days until reaching 0% concentration. One month before behavioural testing, another group was introduced: Adult Standard Water (ASW), in which animals were 3 months old, and received ad libitum commercial chow and tap water. This group served as an age control, and was compared only to group MSW. The whole experiment lasted for 13 months, so that, on sacrifice, animals in middle-aged groups were 16 months old, and those in group ASW were 5 months old. Dietary restriction in restricted groups lasted for the whole period,