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Behavioral effects of dietary cholesterol in rats tested in experimental models of mild stress and cognition tasks

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Abstract

Abnormalities in serum cholesterol levels of patients with mood disorders have been identified in epidemiological studies. However, evidence for an influence of dietary cholesterol on behavioral models is poor. Here, we investigated the behavioral changes of Wistar male rats fed a 2% cholesterol-enriched diet for 2 months in experimental models of depression and anxiety, such as the forced swim test (FST) paradigm and the novelty-induced grooming sampling test (NGT). The correlation between behavioral depression and impaired cognitive capacity was also examined testing rats in the Morris water maze (MWM) task one day after the FST. Different groups of rats fed various dietary regimens, were subjected to acute or repeated treatment (14 days) with clomipramine hydrochloride (50 or 25 mg/kg), diazepam (1 mg/kg) or with the peripheral benzodiazepine receptors (PBRs) antagonist, isoquinoline PK11195 (1 mg/kg) injected intraperitoneally (i.p.). Rats fed the cholesterol-enriched diet showed a significant decrease of grooming score in the NGT and of immobility time in the FST in comparison to animals fed a standard diet. Furthermore, the anxiolytic and antidepressant effects of diazepam and clomipramine were not affected by the different diets. Only after repeated treatment, PK11195 impaired the performance of animals fed a standard diet in the FST, and exhibited an anxiolytic-like profile in animals fed either the cholesterol-enriched or the standard diet. The improved performance in the FST was followed by a better learning performance in the acquisition phase of the MWM. These results suggest that effects of cholesterol-enriched diet on the behavioral reaction of rats in experimental models of mild stress may involve PBRs. They deserve attention in order to clarify the clinical correlation between plasma cholesterol levels and mood disorders in humans.

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1. Introduction

Several studies (for reviews, see Papakostas et al., 2004a; Huang and Chen, 2005) have found low serum cholesterol levels (<160 mg/dl), including triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) in patients with depression, anxiety, co-morbid depression and anxiety, suicidal ideation and current or past suicidal behavior. This finding was not entirely believed to be the consequence of depression-related malnutrition, since confounding by weight change and parameters of nutritional status was excluded in some studies (Morgan et al., 1993; Horsten et al., 1997).

The biochemical relationship between cholesterol and mood disorders is poorly understood. Cholesterol is an essential cell membrane component and may be involved in the development, function and stability of synapse, modulating membrane protein function (Pfrieger, 2003). The brain possesses a high content of lipids, and cerebral cholesterol levels are quite stable. It is not clear if dietary cholesterol directly influences brain cholesterol concentrations (Howland et al., 1998). In general, however, there is evidence that brain lipid content is influenced by dietary lipid intake and alterations in brain lipids makeup, in turn, cause behavioral and other neuropsychic consequences (Oner et al., 1991; Farquharson et al., 1992; Young, 1993; Kaplan et al., 1994). In particular, rats fed dietary cholesterol showed increased cholesterol synthesis in the hippocampus (Koudinov and Koudinova, 2003).

Serotonin (5-HT) neurotransmission in the central nervous system is involved in the pathogenesis of depression. A decrease in brain concentrations of 5-HT and its major metabolite, 5-hydroxyindolacetic acid (5-HIAA) is commonly observed in animals and in patients experiencing stress and depression, suggesting a dysfunction of the 5-HT system (Spreux-Varoquaux et al., 2001). Interestingly, a decrease in plasma 5-HT levels is observed in depressed patients with low levels of plasma lipids (Almeida-Montes et al., 2000). In vitro studies have demonstrated that a decreased cholesterol concentration in neuronal membranes could reduce 5-HT neurotransmission by effects on both pre- and postsynaptic sites (Heron et al., 1980; Engelbergb, 1992; Scanlon et al., 2001). Furthermore, preclinical studies in monkeys have supported the hypothesis of a link between low serum cholesterol concentrations, reduced central 5-HT neurotransmission, and impulsive and aggressive behavior (Muldoon et al., 1992; Kaplan et al., 1994). Recently, major depressive disorder (MDD) patients with high-cholesterol levels exhibited poor response to fluoxetine or nortriptyline treatment, probably due to impairment of 5-HTergic transmission (Sonawalla et al., 2002; Papakostas et al., 2003a,b).

Among other biological actions, the role as a cofactor for signaling molecules has been described for cholesterol. It is a precursor for steroid hormones synthesized in the brain and in the peripheral steroidogenic tissues (Claudepierre and Pfrieger, 2003). For a number of these so-called "neurosteroid", the evidence exists that they are synthesized de novo in the brain from sterol precursors independently from peripheral endocrine sources (Rupprecht, 2003). The biosynthesis of steroids is promoted by activation of peripheral benzodiazepine receptors (PBRs) inducing cholesterol to move from cellular stores into mitochondria with its increased availability for cytochrome *P450* side-chain cleavage enzyme (P450scc), which catalyses the synthesis of pregnenolone (Krüeger and Papadopulos, 1990). The involvement of PBRs in experimental models of anxiety, depression and cognitive deficit has been studied using specific ligands, such as 4′-chlorodiazepam (Ro5-4864), its isoquinoline carboxamide (PK11195) and the 2-phenyl-imidazo[1,2-*a*]pyridine derivatives (Holmes and Drugan, 1991; Bitran et al., 2000; Gavioli et al., 2003; Serra et al., 2004). Furthermore, acute and chronic stress may influence the density of PBRs in various tissues in an opposite manner. Acute stress thus is followed by an increased brain PBRs density and allopregnanolone concentration (Avital et al., 2001; Droogleever Fortuyn et al., 2004; Serra et al., 2004); whereas repeated swim stress (Burgin et al., 1996), repeated foot shock (Drugan et al., 1986) and food deprivation stress (Weizman et al., 1990) lead to a reduction in PBRs density.

In the light of the epidemiological and clinical data described above (for reviews, see Papakostas et al., 2004a; Huang and Chen, 2005), the aim of the present study was to assess the effects of cholesterol-enriched diet on experimental animal models of mood disorders. For this purpose rats fed diet enriched with 2% cholesterol for 2 months as described by Koudinov and Koudinova (2003), were tested in experimental paradigms used to value depression- or anxiety-like behavior as the forced swim test (FST) procedure or the novelty-induced grooming sampling test (NGT), respectively. Since cholesterol is the precursor of steroids through a biochemical process involving PBRs which expression with the steroid synthesis is also influenced by acute or chronic stressors (Burgin et al., 1996; Avital et al., 2001), we also investigated whether the effects of cholesterol-enriched diet may influence the action of PK11195, a PBRs antagonist administered acutely or chronically.

Depression is commonly associated to a cognitive deficit that can be observed as impaired learning and memory and a direct relationship between relief of depression and improvement of the associated cognitive deficit has been described (Sternberg and Jarvik, 1976; Willner, 1984; Bulbena and Berrios, 1993). The 5-HT system plays a role in cognitive processes, particularly in learning and memory (Meneses, 1999) and some antidepressants as tricyclics and selective serotonin reuptake inhibitors (SSRI) enhance learning and memory in animal behavioral tests (Kumar and Kulkarni, 1996; Fujishiro et al., 2002). Since PBRs receptors are also involved in learning and memory processes and at the same time given the relationship between cholesterol and 5-HTergic system (Heron et al., 1980; Holmes and Drugan, 1991; Engelbergb, 1992; Scanlon et al., 2001), these prompted us to assess whether dietary cholesterol and PK11195 may affect the performance of rats in the Morris water maze task after being tested in the FST procedure.

2. Materials and methods

2.1. Animals

Male rats of the Wistar strain (purchased from Charles River, USA) weighing 120–150 g at the beginning of the experiments were used. Rats were randomly assigned to one of the two dietary regimens. Animals were housed two into a cage under standard environmental conditions: constant temperature of 23±1 °C, 60% humidity, 12-h light/dark cycle (lights on between 08.00 and 20.00) with food and tap water *ad libitum*.