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ORIGINAL INVESTIGATION

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Pharmacological evidence of muscarinic-cholinergic sensitization following chronic stress

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Abstract *Rationale:* Although much evidence supports a major role of brain cholinergic transmission in memory consolidation processes, little is known about cholinergic functioning under environmental pressure. *Objectives:* The present experiments were aimed at investigating possible functional adaptation of muscarinic receptors promoted by a chronic stressful procedure in an inbred strain of mice highly susceptible to stress. *Methods:* We tested the effects of post-trial administration of a cholinergic agonist and a muscarinic antagonist on the retention of a passive avoidance task in control animals and compared these effects with those observed following food restriction. *Results:* Food restriction enhanced the facilitatory effects of oxotremorine and reduced the impairing effects of atropine on memory consolidation. *Conclusion:* Our results support the view that chronic sensitization of muscarinic receptors occurs following chronic stress.

Keywords Aversive context · Animal models · Food restriction · Memory · Mice · Dopamine · Acetylcholine

Introduction

Chronic stressful conditions promote a number of adaptive changes in brain functioning, the most known involving brain dopamine (DA) and norepinephrine transmission (Anisman et al. 1993; Cabib and Puglisi-Allegra 1996). The influence of chronic stressful experiences on brain cholinergic functioning, however, has been less investigated.

Moreover, conflicting results have been obtained both by autoradiographic analyses of brain muscarinic receptors and by functional investigation of behavioral responses to pharmacological challenge. Thus, daily exposure to prolonged (2 h/daily immobilization) but not short-lasting (10 min immobilization or forced swim) stress caused a significant increase in the maximal number of muscarinic receptors (Bmax) in several brain areas such as the cortical layers, the CA1 field of the hippocampus and caudate-putamen (Gonzalez and Pazos 1992; Carizzo et al. 1997). Moreover, whilst daily exposure to 2 h immobilization was reported to promote a reduction of the depressant effect of the cholinergic agonist oxotremorine on locomotion (Pullia et al. 1996), 30 min of daily immobilization was shown to enhance the motor effects of oxotremorine (Badiani et al. 1991).

Cholinergic transmission appears to play a predominant role in memory processes (Fibiger 1991 for review). Moreover, the changes in muscarinic cholinergic receptors were observed in brain areas involved in learning and memory (Giordano et al. 1986; Izquierdo et al. 1992; Flood et al. 1998; Baldi et al. 1999; Farr et al. 1999; Setlow et al. 1999). Therefore, memory-related tasks might be the optimal test condition to evaluate functional alterations of brain cholinergic systems through pharmacological challenge.

Mice of the DBA/2 strain are highly susceptible to memory modulation by post-trial administration of cholinergic agents (Castellano et al. 1996). Moreover, this strain of mice appears to be highly susceptible to the effects of environmental pressure (Cabib et al. 2000). Therefore, the following experiments were designed to evaluate the effects of post-trial administration of oxotremorine and atropine on the retention of a passive avoidance task in mice of the DBA/2 strain exposed to a widely used chronic stressful procedure: food restriction.

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Materials and methods

Subjects

Male mice of the inbred DBA/2 strain (Charles River, Como, Italy) were used for these experiments. All mice were purchased at 6 weeks of age. Upon their arrival, animals were housed in fours in standard breeding cages (27×21×13.5 cm) with food and water at libitum on a 12 h/12 h dark/light cycle (lights on between 0700 hours and 1900 hours). Experiments started when animals reached 8 weeks of age. All experiments were conducted according to the Italian national law (DL 116/92) on the use of animals for research.

Housing conditions

Mice housed in groups with free access to food (Food) were used as controls since this is the usual housing condition for laboratory mice. A second group of animals was individually housed and food restricted (85% initial weight) for 13 days and then given food ad libitum for the following 48 h (NFood). The latter paradigm was chosen on the basis of previous results.

In the food-restricted conditions, food was delivered once daily (1000 hours) in a quantity adjusted to induce a loss of 15% of the original body weight. In the free-feeding condition, food was given once daily (1000 hours) in a quantity adjusted to exceed daily consumption (17 g).

Apparatus

Mice were trained on a step-through inhibitory avoidance apparatus similar to that previously described by McGaugh and Landfield (1970). A straight alley was divided into two compartments, one 7.5-cm long and the other 24-cm long, by a partition with a guillotine door. Both compartments were 14-cm high. The floor was 2.5-cm wide and the top 10-cm wide. The smaller compartment was made of white plexiglas. The larger one was made of black plexiglas and was equipped with a removable cover of the same material to allow the compartment to be in darkness. A tensor lamp (60 W, positioned 80 cm above the apparatus) illuminated the side of the small compartment. The floor of the larger compartment consisted of two oblique stainless-steel plates folded at the bottom through which scrambled constant current could be delivered. The shape of the electrified floor ensured the mouse made contact with both plates simultaneously in order to receive the shock.

Procedure

On the training day, each mouse was placed in the lighted compartment, facing away from the dark compartment. When the mouse turned around, the door leading to the dark compartment was opened. When the mouse had stepped with all four paws into the dark side, the door was closed, a foot shock (0.5 mA, 50 Hz, 2 s) was delivered and the latency to step-through was recorded. The mouse was then removed from the apparatus and injected. Retention was tested 24 h later following a similar procedure, except that no shock was administered.

Drug treatment

Oxotremorine (Sigma) and atropine (Sigma) were dissolved in saline (Sal, NaCl 0.9%) and administered intraperitoneally at doses of 0.005 and 0.01 mg/kg or 2 and 3 mg/kg respectively, immediately after training. Saline was used as vehicle (10 ml/kg). The highest doses of the drugs were administered to additional groups of food-restricted mice 120 min after training, as well as immediately after training to other groups of mice that did not receive foot shock. Dosage choice was based on previous experiments (Castellano et al. 1996).

Statistics

Data obtained from animals treated with oxotremorine or atropine were analyzed using two-factor analyses of variance (ANOVAs; housing condition, two levels: Food, Nfood; drug treatment, three levels: Sal, 0.005 and 0.01 mg/kg for oxotremorine and Sal, 2 and 3 mg/kg for atropine). The effects of drug administration 120 min post-trial and following exposure to the apparatus without shock were analyzed using one-way ANOVAs (treatment, three levels: Sal, oxotremorine and atropine). The effects of food restriction on mean step-trough latencies on the training and test days were analyzed using one-way ANOVAs for repeated measures (housing condition, two levels: Food, Nfood as between factor and Training as within factor: Train, Test).

Results

Food restriction did not alter either mean step-through latencies during training (Food=6.5±1.1; Nfood=6.7±0.9) or the acquisition of passive avoidance (Food=75.8±2.8, Nfood=77.1±2.3). ANOVA revealed only a significant main effect of training ($F_{1,14}$=1093.48, P<0.0001).

The effects of drug administration 120 min post-trial and following exposure to the apparatus without shock in food-restricted mice are reported in Table 1. Neither oxotremorine nor atropine affected mean step-through latencies on the test days when administered 120 min post-trial or without shock training.

ANOVA revealed a significant interaction between the housing condition and either oxotremorine or atropine post-trial administration on passive avoidance (oxotremorine: $F_{2,40}$=42.08, P<0.0001; atropine $F_{2,40}$=23.56, P<0.0001). Individual between-groups comparisons performed post-hoc by Duncan's test revealed a significant dose-dependent increase of mean step-trough latencies on the test day in control animals treated with oxotremorine post-trial and a significant decrease in atropine-treated animals. Food restriction promoted a shift on the left of the cholinergic agonist dose–response curve and a shift on the right of the muscarinic antagonist dose–response curve (Fig. 1).

Table 1 Mean (±SEM) step-through latencies on the testing trial of food-restricted mice injected with oxotremorine (OXO), atropine (ATR) or saline (Sal), 120 min after training (120 Post) and in mice not receiving the foot shock on training and administered with drugs immediately following training (No footshock). Note that no significant differences were found

Treatment	120 Post	No foot shock
SAL	74±5.1	4.8±1.6
OXO 0.01 mg/kg	77±4.5	5.0±1.5
ATR 3 mg/kg	76±4.5	5.2±1.5

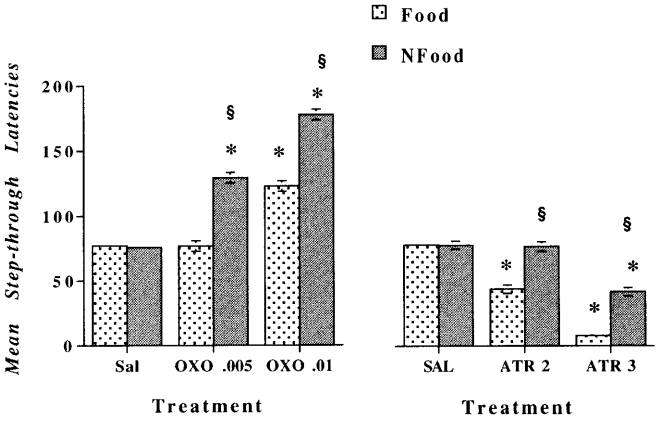

Fig. 1 Effects of post-trial administration of different doses of oxotremorine (*OXO* 0.005 mg/kg and 0.01 mg/kg) and atropine (*ATR* 2.3 mg/kg) or saline (*Sal*) on retention of a passive avoidance task (mean step-through latencies±SEM) in control (*Food*) and food-restricted (*Nfood*) mice. *Significantly different (P<0.01) to *Sal* (Duncan's test). §Significantly different (P<0.01) to *Food* (Duncan's test)

Discussion

Our results demonstrate that chronic stress enhances the facilitatory effects of a cholinergic agonist and reduces the impairing effects of a cholinergic antagonist on memory consolidation. The effects of food restriction on retention performance induced by oxotremorine or atropine in DBA/2 mice are due to an effect on memory consolidation. In fact, they were observed when drugs were given at short, but not long, periods of time after training, which is when the memory trace is susceptible to modulation. Moreover, these effects are not to be ascribed to a rewarding or non-specific action of the drugs on retention performance, as the latencies during the retention test of those mice that had not received a foot shock during the training were not affected by the post-training drug administration.

The present results offer functional support to the view that a chronic stress is capable of promoting sensitization of brain muscarinic cholinergic receptors in specific brain areas. Indeed, the increase in the behavioral effects of the cholinergic agonist and the decrease of the behavioral effects of the muscarinic antagonist strongly suggest an increase in the number of receptors available for transmission. These results are in agreement with the reported increase of muscarinic receptors (Bmax) in chronically stressed rats (Gonzalez and Pazos 1992). To this regard, it should be pointed out that the food-restriction protocol used in the present experiments promote behavioral effects similar to chronic prolonged restraint and produces cross tolerance to the behavioral effects of 2 h restraint (Badiani et al. 1992; Cabib and Castellano 1997; Cabib et al. 2000).

Finally, we have recently shown that food restriction reduces the impairing effects of amphetamine on memory consolidation in DBA/2 mice (Cabib and Castellano 1997). The reduced effects of amphetamine do not seem to depend on stress-induced adaptation of brain DA functioning. Indeed, a number of indirect and direct results indicate facilitation of brain DA transmission in DBA/2 mice following chronic stress (Cabib and Puglisi-Allegra 1996; Puglisi-Allegra and Cabib 1997). Moreover, sensitization to the behavioral effects of amphetamine in different tasks has been demonstrated in food-restricted DBA/2 mice (Cabib et al. 2000). Therefore, desensitization of the behavioral effects of amphetamine on memory consolidation might involve stress-induced sensitization of muscarinic cholinergic mechanisms.

This hypothesis is supported by a number of indirect results. Indeed, the impairing effects of DA agonists on memory consolidation in this strain of mice appear to involve brain muscarinic cholinergic functioning (Gasparri et al. 1997; Castellano et al. 1999). Moreover, anomalous effects of DA agonists and antagonists on hippocampal acetylcholine release have been observed in this strain of mice (Imperato et al. 1996).

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