ROLE OF THE GANGLIONIC BLOCKER CHLORISONDAMINE (CHL) IN MEDIATING STRESS INDUCING IMMUNE RESPONSE IN RATS

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Keywords: stress, chlorisondamine (CHL), immune response

Abstract: The involvement of nicotine acetylcholine receptors (nAChRs) was investigated in acute and chronic stress, influence immune system, with concomitant changes in immune response. Adult rats pretreated with 0.5 ml sheep red blood cells (SRBC) (10^9/ml, Sigma), produce an immune response, and were subjected with chlorisondamine (CHL) (3 mg/kg b.w., i.p.) under acute and chronic stress influence. In the present study, acute stress is a form of short-term stress induced by a single footshock session, and chronic stress is a form of long-term stress induced by multiple and repetitive footshock sessions. 4 days later, we assessed the total number of leukocyte and the antibody titer against SRBC. In summary, we provide the role of ganglionic blocker chlorisondamine in mediating stress-induced immune response in rats.

INTRODUCTION

Chlorisondamine (CHL) is a bisquaternary nicotinic receptor blocker. After systemic administration, it blocks ganglionic nicotinic receptors only temporarily (Clarke et al., 1994), and its effect on nicotinic receptors of the neuromuscular junction is negligible. However, when administered intracerebroventricularly or in a high systemic dose, CHL exerts a remarkably persistent (several weeks) block of central nicotinic responses, examined either behaviorally or in vitro (Reuben et al., 1998; Hefco et al., 2003). The mechanism by which CHL exerts its long-lasting blockade of nicotinic receptors in the CNS remains elusive (Reuben et al., 1998). Peripherally administration of CHL is thus used to evaluate the role of autonomic nervous system in mediating immune response in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200-250 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

Stress procedures and drug administration

Chlorisondamine (CHL) was was purchased from Sigma. All test compounds were dissolved in sterilized pyrogen-free physiological saline. Rats were divided into three groups: 1. saline with acute and chronic footshock treatment; 2. CHL with acute footshook treatment; and 3. CHL with chronic footshock treatment. Prior to footshock treatment all animals were immunized by means of 0.5 ml sheep red blood cells (SRBC) (10^9/ml, Sigma). Prior to the acute footshock stress, rats in group 2 were treated with CHL (3 mg/kg b.w., i.p.) and then were subjected to an intermittent session of footshock: 0.5 mA, during 60s (5s shock, 5s pause) one and three days from the moment of the CHL administration, and then returned to the home cage. Control group rats were subjected to the same conditions and were treated with physiological saline. The rats in group 3 were treated with CHL (3 mg/kg b.w., i.p.) and then were subjected to chronic footshock stress [intermittent session of footshock: 0,5 mA, during 60s (5s shock, 5s pause)] over three consecutive days from the moment of CHL administration, and then returned to the home cage. Control group rats were subjected to the same conditions without CHL administration and were treated with physiological saline.

Four days after the drug administration, whole heparinized blood was collected. The total number of leukocyte was assessed using COULTER® Ac·T™ 5 diff CP blood analyzer and the antibody titer against SRBC were determined by means of Van Djik and Blocksma method.

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student’s “t” test (T- test: Paired Two Sample for Means). $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effects of CHL treatment on the total number of leukocyte and antibody titer

Experimental data were registered after 4 days of continuous CHL administration. The total number of leukocyte decreased significantly in CHL treated-group after three days under acute stress influence (Fig. 1.) and increased significantly under chronic stress influence (Fig. 2.). The antibody titer decreased significantly in CHL treated-group after three days under acute stress influence (Fig. 3.).

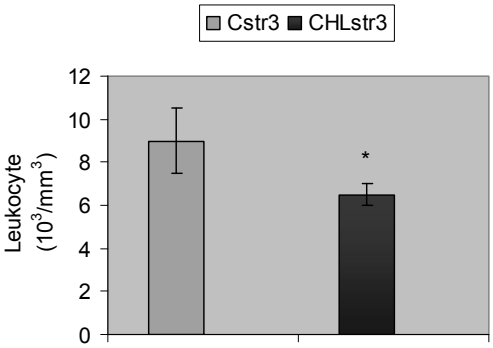

Fig. 1. Changes of the total number of leukocyte in CHL treated rats after three days under acute stress influence and immunization by means of SRBC (10^9/ml). Data are presented as the mean ± SEM; *p<0.04 vs. control group

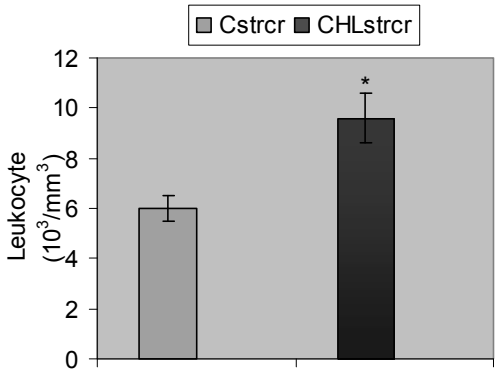

Fig. 2. Changes of the total number of leukocyte in CHL treated rats after three days under chronic stress influence and immunization by means of SRBC (10^9/ml). Data are presented as the mean ± SEM; *p<0.02 vs. control group

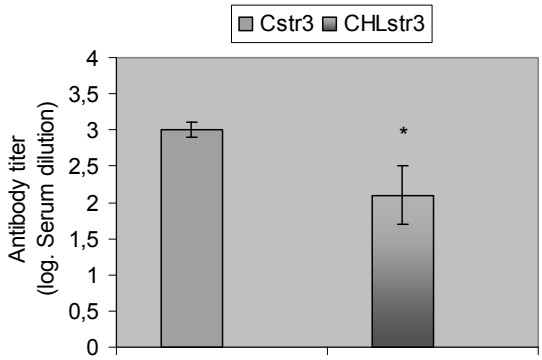

Fig. 3. Changes of the antibody titer in CHL treated rats after three days under acute stress influence and immunization by means of SRBC (10^9/ml). Data are presented as the mean ± SEM; *p<0.05 vs. control group

We have presented the evidence that CHL have a crucial role in mediating immune response in rats during different form of stress. After administration of CHL we observed depressant effects under acute stress (in third day after immunization) and chronic stress. If CHL were to act principally by blocking nicotinic receptors, it would tend to target sites possessing a high tonic level of nicotinic cholinergic transmission.

CONCLUSIONS

We found that the autonomic pathways are involved in mediating the immune response during different form of stress. This involvement consists in a role for nicotinic cholinergic receptors (peripherally or centrally) and for different other nervous pathways like serotonergic, dopaminergic. Future studies may clarify whether these changes represent the direct or indirect consequences of nicotinic receptor blockade, or whether they reflect other, non-nicotinic effects of the drug.

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