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Cholinergic Receptor Sensitivity Following Pharmacological Modulation of the Immune System

KURADY LAXMINARAYANA BAIRY, RAO JYOTHI, VASANTH KUMAR and K BALACHANDRA KUMAR

Department of Pharmacology (K.L.B., R.J., V.K.), and Department of Clinical Psychology (K.B.K.), Kasturba Medical College, Manipal, India.

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ABSTRACT

Objective. To study the effect of immunomodulators on learning and memory and on cholinergic receptor sensitivity. **Methods.** Animals were initially treated for a period of 8 days with cyclosporine/levamisole/ saline. They were then subjected to passive avoidance training and 48 hrs later, the retention test for the passive avoidance task was conducted. Twenty-four hours after the retention tests the animals were subjected to test for cholinergic receptor sensitivity. Oxotremorine was injected intraperitoneally in a dose of 0.46, 1.84 or 4.61 mg/kg. Rectal temperature was recorded in all the animals before administration of oxotremorine and thereafter every 15 minutes for 120 minutes. **Results.** Levamisole exhibited an increased latency to enter the dark compartment as compared to the control rats during the retention test. This increased latency indicates a better retrieval of learned behavior and facilitation of learning and memory processes. On the other hand in the rats treated with cyclosporine, there is a decrease in passive avoidance retention suggesting an adverse effect on learning and memory. The administration of oxotremorine exhibited an enhanced hypothermia in levamisole treated group as compared to control group suggesting hyperactivity of the cholinergic system. On the other hand oxotremorine administration failed to produce significant hypothermia in cyclosporine treated group as compared to control suggesting hypo activity of the cholinergic system. **Conclusion.** Levamisole causes hyperactivity and cyclosporine causes hypo activity of central cholinergic system. Central cholinergic system hypo activity impairs and hyperactivity improves the learning and memory

Keywords: *Cholinergic receptors, Immunomodulators, Learning, Memory*

A substantial body of research indicates that the central cholinergic system plays an important role in the process of learning and memory [1, 2]. Learning and memory process is not an independent process, but is influenced or modified by the immune system [3]. The past decades have witnessed the possible role of central nervous system (CNS) in the regulation of immune function and in turn the feedback from the immune system to the CNS. The interaction between these two systems is suggested to be bi-directional influencing each other in a reciprocal manner [4, 5]. Diminished intellectual functions and motor impairment are seen in patients with AIDS and psychiatric illnesses associated with immunological abnormalities [5]. Viral or cyclosporine induced immunosuppression is found to produce learning and memory impairment [6, 7]. Although a great deal of evidence supports that immunomodulators affect learning and memory, the exact mechanism that underlies this effect is yet to be determined [8, 9].

The present study was undertaken to determine cholinergic receptor sensitivity following pharmacological modulation of immune system.

MATERIALS AND METHODS

Animals

Healthy male inbred albino rats of Wistar strain, weighing 300 ± 10 g, were used in the present study. The animals were housed in-groups of 3 or 4 per cage under an alternating 12-hour light: dark cycle (light on 6.00-18.00 hrs) with free access to food and water in humidity controlled environment. The study was conducted following Institutional ethical committee clearance.

Experimental Design

Animals were initially treated for a period of 8 days with cyclosporine/levamisole/saline. They were then

subjected to passive avoidance training and 48 hrs later, the retention test for the passive avoidance task was conducted. Twenty-four hours after the retention tests the animals were subjected to test for cholinergic receptor sensitivity.

Assessment of Learning and Memory

Passive avoidance test. The two compartment passive avoidance apparatus as described by Buresova and Bures was used [10]. Essentially the apparatus consists of a square box with a floor grid of 50 x 50 cm and wooden walls of 35 cm height. A 100 watts bulb illuminates this box. In the center of one of the walls is an opening of 6 × 6 cm which can be opened or closed using a transparent plexy glass sliding door. This opening leads to a smaller (15 × 15 cm) dark compartment provided with an electrified floor, that can be connected to a shock source (stimulator obtained from Hugo Sachs Electronics, Germany) having a maximum output of 100 mA. Ten animals each from control, cyclosporine and levamisole groups, selected at random were placed individually in an illuminated chamber facing away from the entrance to the dark compartment. The door was closed after the rat entered the dark compartment and 1mA foot shock was delivered for a period of 2 seconds. Then the animal was returned to home cage. 24 hours later each animal was placed again in the passive avoidance apparatus as before for a maximum period of 180 seconds. The latency time required for the animal to enter the dark compartment was measured. Animals not entering the dark compartment within this period received a latency time of 180 seconds. Absence of entry into the dark compartment indicated a positive retention.

Test for cholinergic receptor sensitivity. The activity of cholinergic receptor system was studied using oxotremorine (a synthetic, centrally active cholinomimetic drug that stimulates central muscarinic receptors and used as an experimental tool) [11]. Animals in saline and drugs treated (cyclosporine and levamisole) group were given intraperitoneally 0.46, 1.84 or 4.61 mg/kg of oxotremorine salt (in a volume of 1mg/ml). Thirty minutes before the administration of oxotremorine, methylscopolamine nitrate (Sigma Chemicals, St. Louis, US), and well known to be devoid of central effects was injected to all animals in the dose of 1 mg/kg in order to block the peripheral effects of oxotremorine. Rectal temperature was recorded in all animals just before the administration of oxotremorine (t_0) and thereafter at every 15 min for 120 min. the results were expressed as the mean difference in temperature between (t_0) and other time points.

Drug Schedule

The drugs, dose and number of animals per treatment are given in Table 1. The dose for levamisole and cyclosporine was selected based on previous study [12, 13].

Table 1. The drugs, dose and number of animals per treatment.

Drugs	Dose (mg/kg)	No of animals	Days
Control	Equivolume	10	8
Levamisole	50	10	8
Cyclosporine	25	10	8

Statistical Analysis

Statistical analysis of the variables of latency to enter the dark compartment (among cyclosporine, levamisole and saline treated groups) was done by One-Way Analysis of Variance (ANOVA) and followed by post hoc Scheffé test using the SPSS computer package.

The mean difference in the rectal temperature between time-zero (t_0) and other time points in drug and saline treated groups was subjected to Student's 't' test.

RESULTS

Passive Avoidance Test

There was no significant difference between the different treatment groups (saline, cyclosporine and levamisole) in the initial latency to enter the dark compartment in the passive avoidance paradigm. Therefore any differences seen subsequently are a reflection of differences in the retrieval and are not related to the initial baseline activities. Cyclosporine exhibited a significant reduction and levamisole exhibited a significant increased latency to enter the dark compartment as compared to control (saline) during the retention test ($F(2, 27) = 10.70$ at $p < 0.01$). Longer latency indicates a better retrieval of learned behavior (Table 2).

Test for Cholinergic Receptor Sensitivity

The analysis of the effect of oxotremorine induced hypothermia indicated that levamisole treated animals exhibited an enhanced cholinomimetic-induced hypothermia as compared to control animals. Throughout the period of measurement isolated animals manifested a high sensitivity to all the 3 doses (0.46, 1.84, 4.61 mg/kg), whereas cyclosporine treated animals exhibited a decreased cholinomimetic-induced hypothermia as compared to control animals (Table 3, Table 4, Table 5).

DISCUSSION

The present work was undertaken to study the sensitivity of cholinergic receptors in animals treated with immunostimulant and immunosuppressant drugs. To this end we measured the hypothermia induced by the muscarinic agonist, oxotremorine. We also assessed the passive avoidance memory in immunostimulant and immunosuppressant treated rats, since studies have shown that hypo activity of central cholinergic system impairs and hyperactivity improves passive avoidance

Table 2. Effect of levamisole, cyclosporine and control on latency to enter the dark compartment during training time and retrieval time.

Groups	Latency time during training time. (mean ± SEM)	Latency time during retrieval trial. (mean ± SEM)
Control	30 ± 4.71	166 ±18.95*
Cyclosporine	40 ± 5.58	75 ± 10.87*
Levamisole	32 ± 2.90	241 ± 31.74*

* $p < 0.01$
$F(2, 27) = 10.70$ at $p < 0.01$

Table 3. Thermic response to oxotremorine in control, cyclosporine and levamisole treated animals. Oxotremorine 0.46 mg/kg.

Time (sec)	Mean change in rectal temperature after injection of oxotremorine °C							
	15	30	45	60	75	90	105	120
Saline	0.52±0.05	1.05±0.07	1.66±0.12	2.03±0.12	2.04±0.18	1.14±0.11	0.72±0.10	0.17±0.06
Cyclosporine	0.45 ±0.04	1.01±0.07	1.52±0.06	1.82±0.13	1.39±0.14	0.84±0.09*	0.37±0.08**	0.0±0.09^{NS}
Levamisole	1.01±0.11**	1.83±0.16**	2.56±0.16**	3.63±0.10**	3.59±0.19**	2.57±0.19**	1.24±0.27^{NS}	0.35±0.13^{NS}

The values are expressed as the mean ±SEM and statistical analysis is made at each interval with the student's t-test.
* $p < 0.05$. ** $p < 0.001$. ^{NS} Not significant.

Table 4. Thermic response to oxotremorine in control, cyclosporine and levamisole treated animals. Oxotremorine 1.84 mg/kg.

Time (sec)	Mean change in rectal temperature after injection of oxotremorine °C							
	15	30	45	60	75	90	105	120
Saline	0.52±0.09	0.96±0.16	1.46±0.16	1.95±0.13	2.34±0.21	1.63±0.16	1.06±0.11	0.58±0.07
Cyclosporine	0.39±0.06	0.92±0.06	1.32±0.05	1.61±0.10*	1.29±0.17**	0.86±0.20**	0.27±0.4^{NS}	0.15±0.18^{NS}
Levamisole	1.14±0.11***	1.93±0.13***	2.76±0.16***	3.65±0.21***	4.22±0.21***	3.86±0.24***	2.13±0.11***	0.91±0.12**

The values are expressed as the mean ±SEM and statistical analysis is made at each interval with the student's t-test.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. ^{NS} Not significant.

Table 5. Thermic response to oxotremorine in control, cyclosporine and levamisole treated animals. Oxotremorine 4.61 mg/kg.

Time (sec)	Mean change in rectal temperature after injection of Oxotremorine C°							
	15	30	45	60	75	90	105	120
Saline	0.96±0.17	1.46±0.18	2.26±0.23	3.19±0.22	2.43±0.28	0.66±0.16	0.67±0.16	0.16±0.08
Cyclosporine	0.36±0.04**	0.89±0.09*	1.43±0.11***	2.1±0.18***	2.14±0.17	1.43±0.15**	0.69±0.12^{NS}	0.17±0.08^{NS}
Levamisole	0.81±0.12	1.73±0.17	2.82±0.23	4.31±0.23**	5.07±0.24***	3.70±0.30***	1.92±0.20***	0.72±0.13**

The values are expressed as the mean ±SEM and statistical analysis is made at each interval with the student's t-test.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. ^{NS} Not significant.

memory [13].

In our study we observed that levamisole, a known immunostimulant exhibited an increased latency to enter the dark compartment as compared to the control rats during the retention test. The increased latency indicates a better retrieval of learned behavior and suggests facilitation of learning and memory processes. On the other hand in the rats treated with cyclosporine, there is a decrease in passive avoidance retention suggesting an adverse effect of immunosuppressant on learning and memory.

The administration of oxotremorine, a centrally active cholinomimetic drug exhibited an enhanced hypothermia in levamisole treated group as compared to control group suggesting hyperactivity of the cholinergic system. This supports the earlier finding that hypothermia is related to hyperactivity of cholinergic system [14].

On the other hand oxotremorine administration failed to produce significant hypothermia in cyclosporine treated group as compared to control group suggesting hypo activity of the cholinergic system.

Several studies have suggested that increased cholinergic activity results in improved passive avoidance memory and a decreased cholinergic activity with down regulation of the muscarinic receptors results in deficit in passive avoidance memory [13, 15]. The human and animal observations suggest that the relation of the cholinergic system to memory is due to the plasticity of cortical cholinergic synapses and the cholinergic dependence of limbic structures involved in the memory processes [16].

From the above discussion it is clear that immunostimulants (levamisole) increase cholinergic sensitivity and enhance learning and memory. On the other hand immunosuppressants (cyclosporine) decrease cholinergic sensitivity and adversely affect the cognitive functions. Therefore the altered cholinergic sensitivity observed in this study suggests a possible interaction between the cholinergic system, immune system and cognitive function.

The mnemonic effects of immunomodulators, mediated by central cholinergic system have an important role in the neurocognitive disturbances seen in-patients with Alzheimer's disease, AIDS and such condition related to altered immunological states. However, the implication of the finding is yet to be assessed and has to be validated by a well-defined clinical trial.

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Address correspondence to: Dr. K.L. Bairy, Professor of Pharmacology, Department of Pharmacology, Kasturba Medical College, Manipal, India. E-mail: klbairy@yahoo.com