EFFECT OF NAPHTHENE ACIDS ON LEARNING AND PROTEIN SYNTHESIS IN THE MOUSE BRAIN

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The action of individual components of naphthalan and, in particular, of naphthene acids (NA) on the various functions of organs and systems under normal and pathological conditions is of great theoretical and practical importance for the study of the active principle of naphthalan. The study of the functional state of the CNS is of great importance to elucidation of the mechanism of action of naphthalan in vivo. The key role in the formation and fixation of temporary connections is played by protein biosynthesis [3, 7]. Comparison of the pattern of formation and fixation of temporary connections with that of protein biosynthesis in the brain is therefore one way of discovering the mechanisms of action of naphthalan on CNS function.

The aim of this investigation was to compare the effects of cyclopentane-naphthene acids (CNA), one of the active principles of naphthalan [1], on formation and fixation of temporary connections and on protein synthesis in the brain. The most appropriate method of tackling these problems was to use conditioned reflexes (CR), formed in either one or repeated combinations.

EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 18-22 g. A conditioned active avoidance reflex (CAAR) was formed in a T-maze with electrified floor up to the criterion of five (of six) correct avoidance reactions and evasions. Preservation of the reflexes was tested by repeating their formation up to the same criterion, followed by calculation of the maintenance index. The conditioned passive avoidance reflex (CPAR) was formed by the method of Iarvik and Kopp [8]. According to the conditions of this technique, the animal's move from the lit "safe" compartment of the apparatus into the dark "unsafe" compartment was accompanied by electrodermal stimulation, which continued until the animal returned into the safe compartment. Formation of the reflex thereupon ended. Preservation of this reflex was tested by placing the animal in the safe compartment and recording the length of its stay before moving into the unsafe compartment. The longest length stay of the animal in the lit compartment was restricted to 600 sec, after which the mouse was removed from the apparatus.

Protein synthesis in the brain was investigated with the aid of ^{35}S-methionine with total activity of 1.5 μCi, which was injected intraperiotoneally 1 h before the animals were decapitated. Weighed samples of tissue taken from the cerebral cortex, cerebellum, and brain stem, were homogeneized, proteins were precipitated with 10% TCA, and washed twice with 5% TCA to remove unincorporated label. Lipids were extrated by the usual methods (with ethanol, ethanol-ether, and ether). The radioactivity of the dry protein preparations was determined on PST-100 and DP-100 radiometers by means of BFL-25 end-window counters, with a thin mica window. Radioactivity of the preparations was expressed as specific activity or relative specific activity. CNA were injected in the form of an oily solution intramuscularly in a dose of 150 mg/kg; control animals received the solvent alone. The preparation was injected daily for 10 days and the animals were studied after the last (10th) injection or 10 days after the end of the course of injections.

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Table 1. Effect of CNA on CPAR (M ± m, n = 10)

Group of animals	Duration of stay in lit compartment, sec			
	Control		CNA	
	In initial experiment	On testing preservation of CPAR	in initial experiment	On testing preservation of CPAR
Immediately after end of 10-day course injections	20,30±2,58	401,9	23,60±2,38 (16,2)	454
10 Days after end of 10-day course of injections	16,60±2,07	424,4	23,11±1,81* (39,2)	427

Legend. Here and in Tables 2 and 3, change (in % of control) shown in parentheses; *p < 0.05.

Table 2. Effect of CNA on CAAR (M ± m)

Group of animals	CAAR formation, sec		Preservation, %
	in initial experiment	in reacted experiment	
Immediately after end of 10-day course of injections	24,30±1,40 20,70±1,54	9,85±1,15 6,1±0,85* (38,1)	57,48±4,00 65,50±2,64
10 Days after end of 10-day course of injections Experiment (n = 10)	24,35±1,24 22,30±0,92	10,25±1,02 7,40±0,97* (27,8)	57,17±4,08 66,59±4,13

Table 3. Incorporation of ^{35}S-Methionine into Total Proteins of Mouse Brain under the Influence of a Course of CNA Injec CNA Injections (M ± m, n = 6-8)

Preparation	Cerebral cortex	Cerebellum	Brain stem
	Specific activity, cpm/mg protein		
Immediately after end of 10-Day course of injections Control Experiment	0,664±0,052 0,886±0,083* (33,6)	1,149±0,099 1,264±0,035* (13,0)	0,816±0,068 0,905±0,023 (11,0)
10 Days after end of 10-Day course of injections Control	1,058±0,129* 1,062±0,067 (0,4)	1,608±0,065 1,643±0,222 (2,2)	1,225±0,159 1,233±0,123 (0,6)
	Relative specific activity		
10 Days after end of 10-day course of injections	1,69±0,31 2,66±0,69* (57,4)	2,54±0,39 2,88±0,77* (13,4)	2,08±0,41 2,64±0,57* (27,4)
Experiment Control (n=5)	1,17±0,19 1,23±0,21 (5,1)	2,40±0,55 2,39±0,27 (0)	1,19±0,16 1,25±0,22 (4,9)

Preservation of the reflexes was tested in all cases 1 h after their formation. Statistical analysis was carried out by Student's or nonparametric tests [2].

EXPERIMENTAL RESULTS

Injection of CNA into mice causes changes in the formation and fixation of temporary connections (Table 1). A tendency was observed for the length of the animals' stay in the lit compartment of the apparatus to be increased in the initial experiment. Under the influence of the preparation a definite tendency also was observed toward improvement of preservation of CPAR.

It might be supposed that this tendency is the result of the action of the same factor as causes the increase in the legnth of the animals' stay in the lit compartment of the chamber in the initial experiment. In that case, however, the increase in the duration of stay in the lit compartment in the repeated experiment (i.e., when testing preservation of CPAR) ought to be proportional to its increase in the initial experiment. However, in our experiments it was not always observed to be proportional: 10 days after the end of the course of CNA injections the length of stay in the lit compartment increased in the case of the experimental animals compared with the controls by 39.2%, and remained virtually unchanged in the repeated experiment. This suggests that the preparation exerts its effect both on processes lying at the basis of temporary connection formation, and also on processes related to reinforcement of the connections and their subsequent recall.

The results of the CAAR experiments confirm this conclusion (Table 2). Under the influence of CNA the rate of CR formation increased. At the same time there was a clear tendency for preservation of the established reflexes to be improved. Ten days after the end of the course of injections of CNA the rate of CR formation in the experimental animals became the same as in the control, although a tendency was still observed for their preservation to improve, which could indicate a difference in the physiological mechanisms of formation and consolidation (and preservation) of temporary connections.

To assess the effect of CNA on protein synthesis in the brain, incorporation of ^{35}S-methionine into total proteins of the cerebral cortex, cerebellum, and brain stem was studied. The data in Table 3 show both the specific activity and the relative specific activity (allowing for radioactivity of the TCA supernatant). In both cases the effect of CNA was observed only immediately after the end of the course of injections. Ten days after the end of the course the values for incorporation of ^{35}S-methionine into total brain proteins were virtually identical with the control values. When the data in Table 2 are compared, the stronger tendency for the values of ^{35}S-methionine incorporation into proteins to be increased, when expressed as a ratio to activity of the supernatant, will be noted. This means that CNA activates protein synthesis proper in the brain irrespective of any changes in the precursor pool.

Physiologically active CNA thus have a marked stimulating effect on activity of the higher levels of the CNS. This stimulation is manifested as the more rapid formation and improvement of fixation of temporary connections and of some degree of activation of protein synthesis in the brain. The results as a whole suggest that CNA give rise to two kinds of effects: relatively temporary, in the form of changes in the momentary functional state of higher levels of the CNS, with corresponding changes in the rate of CR formation and effects of a more prolonged character, possibly connected with activation of synthesis of total and (or) specific proteins in the brain and the formation of a stable temporary connections. It is probable that a definite role in the realization of these effects is played by the effect of CNA on biosynthesis of prostaglandins and cAMP (universal regulators of cell functions [4]), for we know that a change in the state of this system affects the functional activity of the CNS [5, 6].

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