PHYSIOLOGY

Effect of Immunization Against Alcohol Dehydrogenase on the Behavior of White Rats

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Active immunization of white rats with alcohol dehydrogenase (horse ADH-1), a major ethanol-metabolizing enzyme shown to cause considerable and long-term inhibition of alcohol consumption, did not have adverse effects on their behavior. Rather, the rats showed enhanced motor and orienting-exploratory activities, developed conditioned responses (with both positive and negative reinforcements) more readily, and spent less time in a state of immobilization in the forced swimming test as compared with nonimmunized controls.

Key Words: *alcohol dehydrogenase; immunization; alcoholism; behavior; rats*

We have shown previously that immunization of rats with a major ethanol-metabolizing enzyme, alcohol dehydrogenase (ADH), and in particular with the isozyme horse ADH-1, results in a marked and long-term inhibition of alcohol consumption [1,3,4,7]. The mechanism of this inhibition possibly involves the production of antibodies to ADH-1 and alteration of its activity in the liver and some other tissues. The method we used has the advantage of enabling ADH activity to be regulated *in vivo* in a direct and selective manner, whereas the commonly used ADH inhibitors (pyrazole and its derivatives) produce a wide range of concomitant and at times unfavorable physiological effects [5].

However, if active immunization with ADH is to be successfully used for eliminating the main clinical manifestations of alcohol dependence in experimental animals, the effects of such immunization on other behavioral parameters must be thoroughly examined. The present study was undertaken with this aim.

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MATERIALS AND METHODS

The study was conducted on random-bred white male rats weighing 180-250 g. ADH (EC 1.1.1.1) from horse liver (Sigma) was used for their immunization. The enzyme was injected subcutaneously into four points on the back three times at 7-10-day intervals, the first two injections being done in a mixture with complete Freund's adjuvant in the 1:1 ratio. Three different ADH-1 doses were used: 25, 50, and 150 μg per animal in a volume of 0.2 ml. These doses were chosen because in our previous studies they had been found to produce the intended effect, namely strong inhibition of the craving for ethanol [1,2,4]. Control rats received physiological saline in the same volume. In some tests, the enzyme was injected into the caudal vein (100-150 μg per rat).

Motor and orienting-exploratory activities were evaluated using a device by which the horizontal and vertical components of motor activity can be

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recorded separately, as can be orienting and exploratory activities, by noting the number of times the animal investigated the upper and lower holes of the test chamber.

The food-procuring response was developed in the rats in a Y-shaped maze containing two feeding troughs, in one of which they could receive reinforcement as described previously [2].

In the learning of conditioned active avoidance responses (CAAR), the presentation of a conditioned stimulus (light) was completed with an electric shock delivered through the grid covering the chamber floor. The shock could be avoided if the rat was able to leap up in time onto the shelf on the wall. On each of the three days of conditioning, the number of avoidance responses per 10 presentations of the conditioned stimulus was recorded.

The tendency of rats to develop a depression-like state in the forced swimming test was evaluated by the classical procedure of Porsolt [6]. In this test, periods of immobility and of active swimming and passive floating were registered.

Sensitivity to the narcotic dose of ethanol was assessed by noting the length of time the rat was lying on its side after intraperitoneal administration of a 25% alcohol solution at a dose rate of 4.5 g/kg body weight.

The results were treated statistically using Wilcoxon's and Fisher's tests.

RESULTS

Alcohol-untreated rats immunized subcutaneously with the heterologous ADH displayed, in most instances, heightened motor and orienting-exploratory activities. These effects were dose-dependent, but no direct correlation between their magnitude and the ADH doses used was observed. The ADH also stimulated these activities when injected intravenously.

The immunization of nonalcoholized rats did not significantly affect the elaboration of the maze reflex (the food-procuring habit), although a tendency toward improved learning was apparent. In contrast, the rats that had been made physically dependent on alcohol by being given a free choice between a 15% ethanol solution and water for 6 months and had consumed considerably quantities of ethanol were found to develop the food-procuring habit much more readily 2 months after immunization with the heterologous ADH was started. Thus, the number of their correct approaches to the food-containing trough was higher than in the control group on all three days when such rats were tested and particularly on days 2 and 3 (Fig. 1).

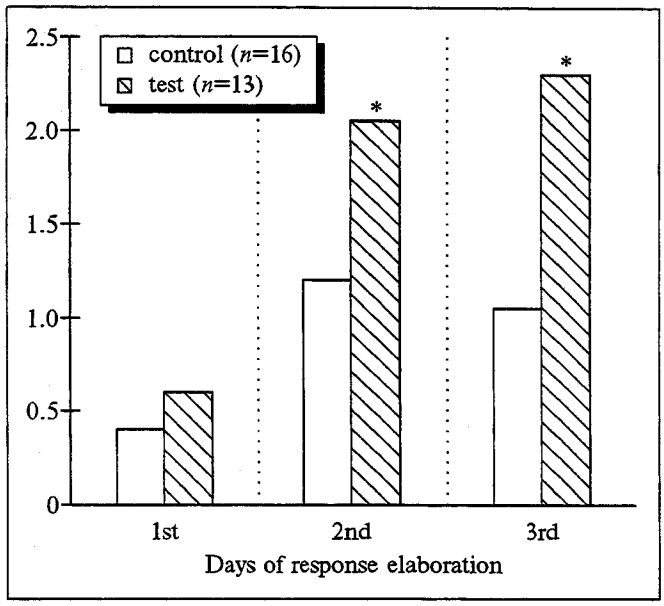

Fig. 1. Effect of immunization with ADH on the elaboration of the food–procuring response in rats. Ordinate: number of correct approaches to the feeding trough (mean values per group). Here and in Fig. 3 an asterisk denotes significant difference from the control group at $p<0.05$.

One-and-a-half months after the start of immunization the rats were able to learn CAAR much more easily, especially those immunized with the smaller of the two ADH doses used (50 as opposed to 150 μg). On days 2 and 3 of conditioning, the number of avoidance responses by rats immunized with 50 mg was 3 times or more

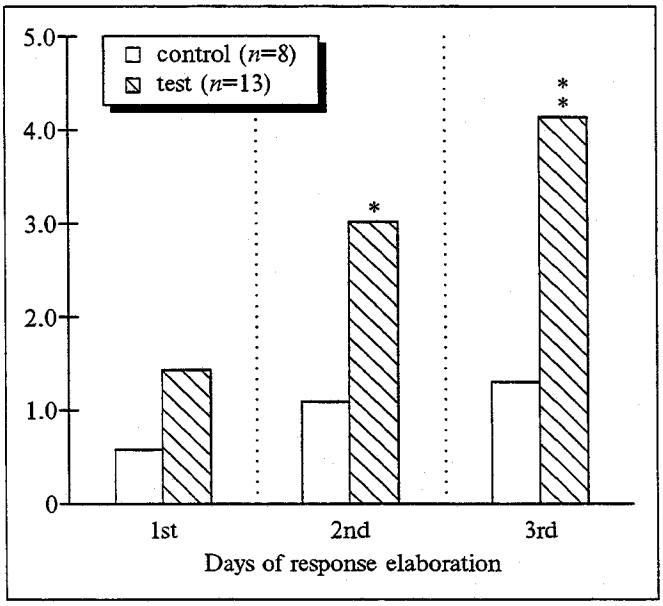

Fig. 2. Effect of immunization with ADH on the elaboration of the conditioned active avoidance responses in rats. Ordinate: number of avoidance responses (mean values per group). One and two asterisks denote significant difference from the control group at $p<0.05$ and $p<0.01$, respectively.

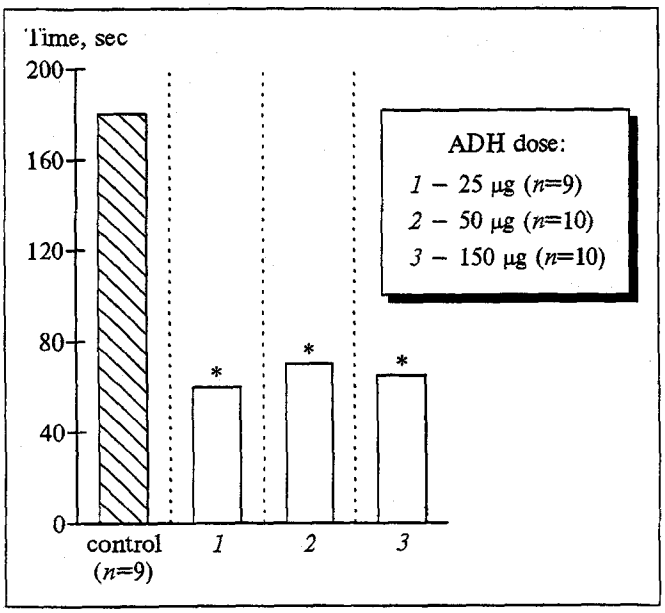

Fig. 3. Effect of immunization with ADH on total immobilization time in rats.

higher than in the control group (Fig. 2). The enzyme also facilitated the learning of CAAR significantly when administered by the intravenous route ($p<0.01$).

In the forced swimming tests, significantly reduced (almost threefold) periods of immobility and passive floating (total immobilization time) were observed 2 to 5 weeks after the start of immunization in all three test groups (those immunized with 25, 50, or 150 μg); the results of tests carried out after 5 weeks are presented in Fig. 3. The baseline values of immobilization time (1 week before the start of immunization) were very similar in all groups.

Reductions in total immobilization time (as a result of increases in the period of active swimming) were also shown by rats injected with the enzyme intravenously. Since these reductions were only observed 20 or more days postinjection, they may be associated with immunization with the heterologous ADH. Immunoassays revealed the presence of anti-ADH antibodies in the blood serum.

Much the same results were obtained with immunized rats that had developed a strong alcohol dependence: the immobilization times in this group were nearly 50% lower than in the control group of "alcoholics."

The effect of immunization on the duration of ethanol-induced narcosis (sleep) in nonalcoholized rats at different times after the start of immunization with two different doses of the enzyme is shown in Table 1. In all cases the immunized rats slept longer than the controls. Similar prolongations of ethanol-induced narcosis were observed for immunized alcohol-dependent rats as well as for native rats at the time when anti-ADH antibodies were detected in their sera after intravenous ADH administration.

One possible reason for the observed effect could be lowered activity of endogenous ADH in the liver or other tissues of immunized rats [7]. It is believed that the duration of ethanol-induced narcosis is inversely related to the degree of alcoholic motivation, and this view is supported by our findings: alcohol consumption by rats following active immunization or intravenous ADH administration was considerably lower than that by control animals.

Thus, the use of ADH, far from producing adverse behavioral effects, even improved some types of learning, which suggests that inverse immunoregulation may be a promising method for the treatment of alcoholism.

TABLE 1. Effect of Immunization with ADH on Sleep Duration (in min) in Rats as Observed in the Ethanol Narcosis Test ($n = 10$)

Time after start of immunization	Control rats	Rats receiving ADH in a dose	
		50 μg	150 μg
Day 11	105	185	205*
Day 17	145	245*	185
Day 28	157	250	265**

Note. One and two asterisks denote $p<0.05$ and $p<0.01$, respectively, relative to controls.

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