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EXPERIMENTAL ARTICLES

Induction of Autoimmunity against Endogenous Neuroregulators Isatin and Cholecystokinin as a Method of Modeling and Correction of Depressive Behavior

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Abstract—Long-term depressive behavior was modeled via immunization of white rats with isatin (an endogenous MAO inhibitor) covalently bound to a carrier antigen. The immunization of rats against isatin and several exogenous MAO inhibitors (pargyline and deprenyl) resulted in long-term (2 months) depressive-like behavior with elements of anxiety and an increased activity of MAO in the brain. In contrast, immunization against endogenous inductors of anxiety (cholecystokinins 4 and 3) induced prolonged antidepressant and anxiolytic effects. The authors discuss the advantages of the immunochemical approach, both to modeling of depression and pathological anxiety, and to their long-lasting correction.

Key words: inverse immunoregulation, depression, isatin, cholecystokinin 4

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INTRODUCTION

The development of new models of pathological depression and anxiety is closely associated with the understanding of their neurochemical mechanisms. As well, adequate models are important prerequisites for new therapeutic methods. Numerous contemporary models of depression frequently suffer from serious disadvantages, such as transient effects and/or the necessity of repetitive exposure to depression-inducing factors. Only the models associated with surgical traumatization of specific brain regions or the models that use specific animal lines are free of these disadvantages [1–3 and others]. However, one can hardly regard traumatic models as adequate. On the other hand, the search for the genetic basis of depression with the use of specific lines of laboratory animals is attractive, but its epigenetic mechanisms remain obscure. A large body of information has been accumulated and numerous hypotheses have been proposed during the past few years about the involvement of neuroimmune processes in the pathogenesis of depression [4, 5, etc.]. In searching for new methods of modeling and suppressing depression, we attempted to use so-called inverse immunoregulation that consists in the induction of production of autoantibodies to endogenous chemical regulatory agents [6–8].

We have previously shown that long-term depressive states can be induced in experimental animals via induced production of autoantibodies to Sydnophen (a psychostimulant with an additional antidepressant effect), pargyline and deprenyl (antidepressants and exogenous inhibitors of monoamine oxidase) [9–11]. This immunization resulted in the production of antibodies to pargyline and deprenyl accompanied by an increase in the activity of monoamine oxidase (MAO) and by changes in the levels of biogenic amines. Biochemical changes were correlated with deep and long-term changes in the behavior of rats which are characteristic of a depressive state (a decrease in exploratory activity, increased immobilization time in the Porsolt test, and symptoms of anxiety).

In this aspect, however, endogenous regulatory substances deserve even more attention than exogenous synthetic inhibitors of MAO. Tribulins are important endogenous agents that restrict the activity of brain monooxidases and, consequently, the development of stress, anxiety, and depression [12]. Isatin (2,3-dioxyindol) is the main active component of tribulin B. Here, we present the results of our attempts to model depressive behavior in rats via immunization with isatin covalently bound to a carrier antigen. We also discuss the advantages of an immunochemical approach to the long-term correction of depressive behavior based on the immunization of rats with conjugates of endoge-

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Abbreviations: BSA, bovine serum albumin; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HIAA, hydroxy-3-indoleacetic acid; 5-HT, 5-hydoxytryptamine; HVA, homovanillic acid; MAO, monoamine oxidase; CCK-3 and CCK-4, cholecystokinins 3 and 4.

Table 1. Parameters of immunological analysis after immunization of the rats with the conjugates of isatin or deprenyl

Group	Titers of antibodies	
	to deprenyl	to isatin
Control	160	160
Immunized against deprenyl	6400	6400
Immunized against isatin	12800	12800

Table 2. Activity of MAO (nmol of NH_4^+/min) after immunization of the rats with the conjugates of deprenyl or isatin

	Liver	Brain
Control	0.166 ± 0.015	0.1 ± 0.02
Deprenyl	0.192 ± 0.007 (116%)	0.153 ± 0.011 (153%) $p < 0.05$
Isatin	0.145 ± 0.009 (87.3%)	0.186 ± 0.02 (186%) $p < 0.01$

nous anxiety factors, and cholecystokinins 3 and 4 (CCK-3 and CCK-4).

EXPERIMENTAL

The study was performed with white Wistar rats (no less than 10–12 animals in each control and experimental group).

Immunization of Rats

The isatin (an endogenous inhibitor of MAO) and Deprenyl (*N*-dimethyl-*N*-(2-propyl)-benzeneethanamine hydrochloride, ICN Biomedical, United States) covalently bound to bovine serum albumin (BSA was used as a carrier antigen) were used for the immunization of rats. The conjugates were synthesized using carbodiimide-1 (*N*-ethyl-*N*'-dimethylaminopropyl carbodiimide hydrochloride) taken as coupling reagent at a molar ratio of 1 : 100. Rats were injected with 600–800 μg of the conjugate per 1 kg of the body weight three times into four points of their backs at 7- or 8-day intervals. The first and second injections were performed with Freund's adjuvant (ICN Biomedical, United States) added at a 1 : 1 ratio as an immunostimulant. Control animals were injected with a mixture of the adjuvant and saline.

Immunological Analysis

Serum samples of control and experimental animals were tested by ELISA using the phosphate buffer (pH 7.5).

Biochemical Studies

The MAO activity was evaluated in mitochondria isolated from brain and liver extracts of control and experimental rats by differential centrifugation in 0.25 M sucrose. This was determined according to the accumulation of a colored (450 nm) product generated by the oxidation of *p*-nitrophenylethylamine at 25°C. The reaction mixture contained 950 μl of 50 mM phosphate buffer (pH 7.4), 25 mg of Triton X-100, 50 μl of the mitochondria suspension, and 0.1 mM *p*-nitrophenylethylamine. Change in the absorbance by 0.01 corresponded to the generation of 0.33 nmol of NH_4^+.

The levels of striatal monoamines and their metabolites were determined by HPLC with electrochemical detection.

Behavioral tests were performed 3 or 4 weeks after the start of the immunization and later. The following tests were used.

1. The rat's predisposition to depressive behavior was determined using the 10-min Porsolt test of forced swimming. A computer recorded the durations of active (intensive strokes with all paws) and passive (weak strokes with hind paws) swimming and immobilization (immobility) of the animals.

2. The components of anxiety and fear in the behavior of rats were evaluated by a 5-min test in the elevated plus maze. The number of entries into the open arms and the time spent there, the total number of transitions, risk behavior, the number of immobility events and their duration, and other parameter were recorded.

Statistical analysis was performed with the Wilcoxon–Mann–Whitney U-test and the Student's *t*-test, using Microsoft Excel and Statistica software.

RESULTS AND DISCUSSION

Immunological Effects

Immunization with the isatin–BSA conjugate resulted in the production of a rather high and persistent concentration of antibodies to isatin with a mean titer of 1 : 12 800 (in control rats it was 1 : 160). Table 1 shows that immunization against isatin resulted in a higher titer than that against the deprenyl exogenous regulatory agent. The differences in titers between control and experimental animals were statistically significant ($p < 0.001$). Tests for cross-reactivity showed that all the antibodies raised against deprenyl were able to react with isatin, and vice versa, all the antibodies to isatin reacted with deprenyl.

Biochemical Effects

Table 2 shows a reproducible increase in the activity of MAO in the brains of immunized rats. This was more significant in rats immunized against isatin. These changes in the MAO activity resulted in a significant decrease in the striatal levels of serotonin and its metab-

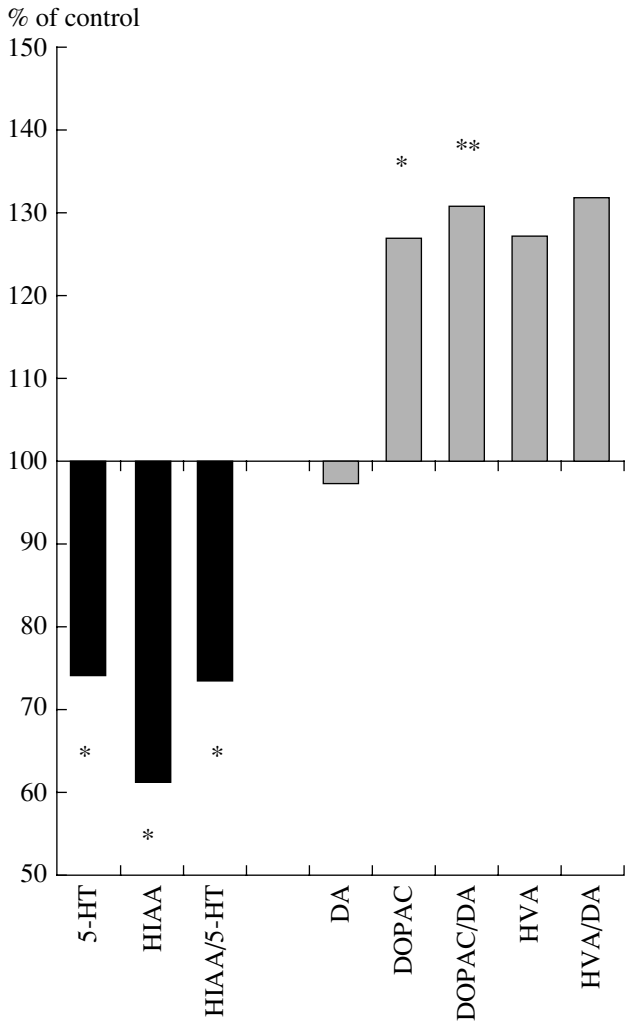

Fig. 1. Biochemical changes in the striatum of the rat brain after immunization with the conjugate of isatin with BSA. The ordinate shows the levels of serotonin, dopamine, and their metabolites in the experimental rats relatively to the control animals (%). *$p < 0.05$, **$p < 0.01$.

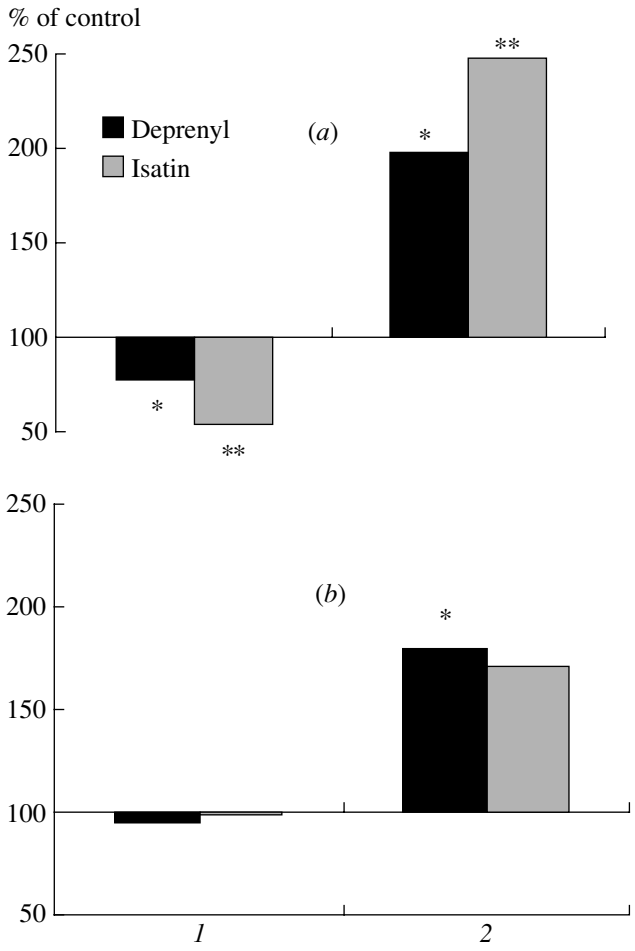

Fig. 2. The parameters of forced swimming: (*a*) after immunization of rats with the conjugates of deprenyl or isatin; (*b*) after administration of fluoxetine to immunized rats. The ordinate shows the durations of the active swimming and the immobility of experimental rats relatively to the control animals (%). (*1*) Active swimming; (*2*) immobility. *$p < 0.05$, **$p < 0.001$.

olites in the brains of immunized rats. This was not accompanied by any significant changes in the levels of dopamine, although the levels of its metabolites were higher than in control animals (Fig. 1).

Behavioral Effects of Immunization

Figure 2a illustrates the results of the Porsolt's forced swimming test used to determine depressive components in the behavior of the animals. The rats immunized against the conjugated isatin displayed a highly significant decrease in active swimming times ($p < 0.001$) in comparison with the control group. Most importantly, the increase in the immobilization time was also significant ($p < 0.001$). Forced swimming tests were performed twice, at early and late stages of the immunization with conjugated isatin (an endogenous inhibitor of MAO) and deprenyl (an exogenous inhibitor). The results indicate that the former agent induced a more pronounced depression of behavioral activity than the latter.

Decreases in orientation–exploratory activity and depressive components were also observed in tests with the elevated plus maze. A decrease in the total number of transitions, a significant increase in the initial time lag before the beginning of movements (231% of control, $p < 0.05$), and prolonged immobility (147.4%, $p < 0.05$) were observed. In addition, the immunized rats exhibited changes in the parameters characteristic of anxiety and fear: less frequent entries into the open arms of the maze and shorter time spent in them (40 and 16.9% of control, respectively).

Treatment of the rats immunized with Deprenyl and isatin with the Fluoxetine antidepressant, administered in an acute mode (three times a day at a dose of

Table 3. Parameters of the forced swimming test after immunization of the rats with the conjugate of CCK-4 with BSA

	Active swimming	Immobilization
Control	435.09 ± 35.34 s	170.36 ± 24.75 s
CCK-4	533.80 ± 17.29 s $p < 0.001$	56.20 ± 10.26 s $p < 0.001$

20 mg/kg) prolonged the active swimming time practically to the control level (Fig. 2*b*), but had almost no effect on the immobilization time. In contrast, the chronic administration of fluoxetine, (for a week) not only prolonged the active swimming time, but also decreased the immobilization time (however, not to the control level). The fluoxetine-induced partial removal of the symptoms of depression, especially in rats immunized against isatin, indicated that immunization against MAO inhibitors resulted in a deep and stable depression.

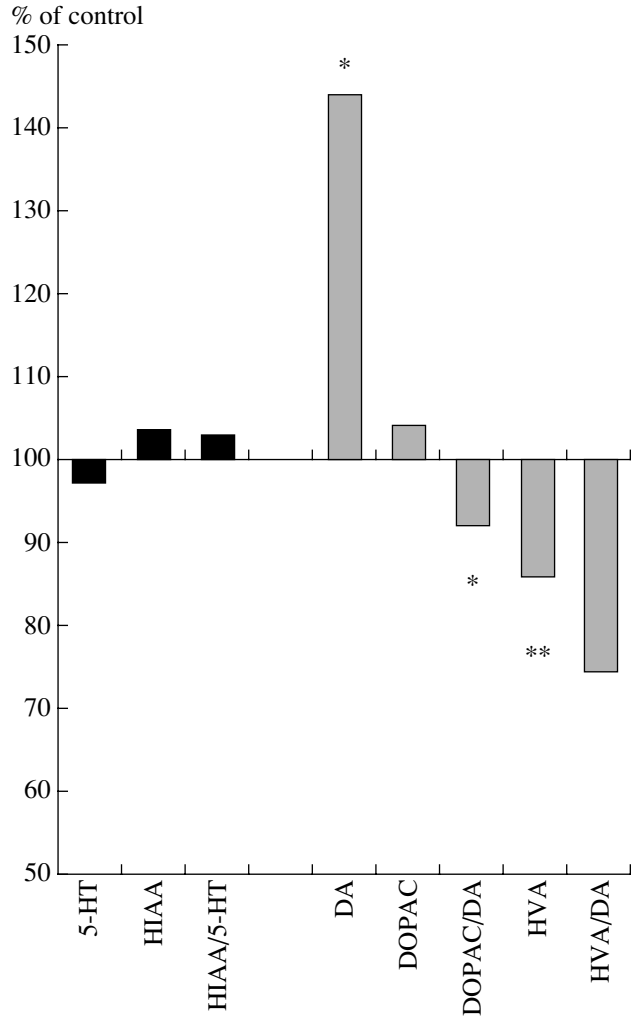

Fig. 3. Biochemical changes in the striatum of the rat brain after immunization with the conjugate of CCK-4 with BSA (% of control). The ordinate shows levels of serotonin, dopamine, and their metabolites in the experimental rats relatively to the control animals (%). *$p < 0.05$, **$p < 0.01$.

Hence, our results demonstrate that the induction of antibodies caused by the active immunization of rats against endogenous (isatin) and some exogenous (pargyline and deprenyl) inhibitors of monooxidases induces a depression-like behavior with elements of anxiety. The duration of such a condition exceeds two months, and it can be partially relieved with the fluoxetine antidepressant. Therefore, this isatin-based model can be recommended for tests of the efficacy of new antidepressants.

Various studies have demonstrated that the blood tests of patients with psychic illnesses detected significant levels of antibodies specific to several antigens and haptens of the brain. Rats with experimental dopamine-deficit-dependent depressive syndrome were shown to produce antibodies to serotonin and dopamine [13]. One of the main reasons for this long-term depression could possibly be an autoimmune process, such as the generation of antibodies to endogenous regulators of the MAO activity, including isatin or isatin-containing tribulin. The prolonged circulation of such antibodies is able to suppress the isatin inhibition of MAO and intensify the cleavage of serotonin and dopamine, which is evident from the biochemical changes in the brain found in this study. It has now been shown that antibodies can cross the blood-brain barrier and penetrate into the brain (although only if their residence time in the blood is sufficiently long) [14–16]. In this context, further studies of the level of autoantibodies to isatin could be useful for the evaluation of the severity of depression and /or its latent form.

Induction of long-term depression by immunization against inhibitors of MAO, such as pargyline, deprenyl, and, especially, isatin, is also important for the correction of manic and aggressive states. However, the use of neuroimmunological approaches to the long-lasting correction of depression appears to be especially important. In this context, our results obtained with rats immunized against CCK-4 and CCK-3 (the endogenous anxiety-inducing factors) are of special interest, because they revealed not only an anxiolytic effect, but also an antidepressant effect, i.e., changes opposite to those observed after immunization against the MAO inhibitors [17, 18]. In the forced swimming test, such rats displayed an increased active swimming time in comparison with the control group, but the time of immobility significantly decreased (Table 3).

Similar to the case of behavioral changes, the biochemical changes after immunization with CCK-4 and CCK-3 were opposite to the changes observed after immunization against the MAO inhibitors (Fig. 3). A significant increase in the level of dopamine (DA) and a decrease in the levels of its metabolites, such as homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), as well as a decrease in the HVA/DA

and DOPAC/DA ratios, were observed. The level of 5-hydoxytryptamine (5-HT) remained unchanged.

The methods of immunosuppression of drug addictions and contraception are already used in clinical practice [19, 20, etc.] and, thus, successfully demonstrate the potential of the use of the described immunochemical approaches, not only for depression modeling, but for therapy as well.

CONCLUSIONS

As a whole, all these results allow us to recommend our immunochemical approach for the regulation of behavior in not only modeled pathological depression and anxiety, but for a long-lasting correction of these states as well.

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