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The Influence of Agonists and Antagonists of Dopaminergic System on the Formation of Audiogenic Seizures in Rats During the Ethanol Abstinence Period

Wpływ agonistów i antagonistów układu dopaminergicznego na powstawanie drgawek audiogennych u szczurów w okresie abstynencji etanolowej

There have been numerous studies on the influence of ethanol on neurotransmitters, with conflicting results for the individual transmitters. It also refers to dopamine Hunt and Majchrowicz (8) have found that dopamine circulation lowers after a prolonged administration of ethanol. The results of other experiments point out that such a long-lasting activity of ethanol has no influence on dopamine circulation (1). Tabakoff and Hoffman (13) indicated that dopamine synthesis is lowered during the symptoms of ethanol abstinence. Griffiths et al. (7) as well as Wajda et al. (15) have proved that the level of dopamine in the striatum occuring at ethanol abstinence increases and comes back again to its initial value after 18 or 48 hrs. Fadda et al. (6), while examining the prolonged influence of ethanol upon dopamine metabolism, has shown that after 24 hrs since the stop in ethanol administration, there has been an increase of dopamine level in the rat's frontal lobe of *cortex cerebri*. In other structures of the brain the dopamine level has not been changed. Darden and Hunt (5) obtained the results showing a considerable decrease of dopamine liberation in the striatum during abstinence periods.

In view of the biochemical research it is difficult to estimate explicitly the role of dopaminergic system in the appearance of dependence and symptoms of ethanol abstinence in the experimental animals. Some behavioral obsęrvations carried out on animals during the ethanol abstinence suggest that the prolonged administering of ethanol brings about hypofunction of central dopaminergic neurons.

In this paper we investigate the influence of the chosen agonists and antagonists of the dopaminergic system on the appearance of audiogenic seizures in rats during the ethanol abstinence period.

METHODS

The experiments were performed on male rats of Wistar type with the body mass of 200—220 g each. The animals were locked in cages (5 in 1 cage) at standard laboratory conditions. They had a free access to water and granulated food (Bacutil, Motycz).

Ethanol was administered intragastrically (i. g.) as 40% water solution (v/v) at 6.00 a.m., 2.00 p.m., and 10.00 p.m. for 5 succeeding days. Dependence on ethanol began by administering ethanol to all the rats in dose of 5 mg/kg. The subsequent doses of ethanol depended on the state of a given animal and were 4, 3, 2 or 1 g/kg. Throughout all the period of dependence the animals were receiving the 5% (v/v) solution of ethanol instead of water. There was not any estimation of the amount of ethanol drunk by the animals. The control animals were receiving distilled water i.g. instead of ethanol.

The final administration of ethanol was performed on the 6th day of the experiment at 6.00 a.m. The animals were divided into 11 groups with 8—13 rats in each. Five of those groups made up the control part. The animals in the remaining groups were given one of the following substances intraperitoneally (i. p.): Bromocriptine mesylate (Sandoz, Basel), Amantadine HCl (EGIS, Budapest, Hungary), Sulpiride (Podravka OOUR Belupo 2, Koprivnica, Yugoslavia), Metoclopramide HCl („Polfa", Starogard) and Haloperidol („Polfa", Warszawa). Their influence on the appearance of audiogenic seizures was investigated. These observations were made between the 13th and 14th hrs of ethanol abstinence. The animals were placed separately in glass cylinders. The seizures were caused by a sound of an electric bell with the volume of about 100 dB, placed in the cylinder. The sound stimulus lasted no longer than 60 s.

Bromocriptine is doses of in doses of 2.5 and 10 mg/kg was given 1 hr before the test. Amantadine was applied in a dose of 25 mg/kg 30 min before the appearance of audiogenic seizures. Sulpiryd in a dose of 10 mg/kg was administered 40 min before the sound stimulus. Metoclopramide of 10 mg/kg was injected 1 hr before the test. Haloperidol was applied in a dose of 0.5 mg/kg 30 min before the observation.

Bromocriptine mesylate was administered as the Tween 80 suspension. The remaining substances were dissolved in physiological salt. The control rats were receiving physiological salt i.p. The obtained results were worked out statistically with the help of test X^2 and Yates's modification.

RESULTS

The symptoms of ethanol abstinence in the form of audiogenic seizures was assessed in rats after 13—14 hrs since the last administration of ethanol. The examined rats, too, had other symptoms of abstinence like piloerection, head shaking, body shivering, tail stiffness, exophthalmos and touch sensitivity. While performing each of the experiments, the percentage of animals in the control groups reacting to a sound stimulus with seizures was no less than 75.

Bromocriptine in doses of 2.5; 5 and 10 mg/kg slightly increased audiogenic seizures. The activity of bromocriptine was not dependent on the applied dose (Table 1). A different result was obtained after administration of amantadine. Statistically it really suppressed audiogenic seizures in dependent rats (Table 1). Metoclopramide and sulpiride did not have any influence on audiogenic seizures. Similarly, haloperidol did not affects the appearance of audiogenic seizures in any essential way. After its administration, the percentage of animals displaying these seizures was only slightly increased (Table 1).

DISCUSSION

The results obtained in the present study demonstrated that amantadine administered in doses of 25 mg/kg really inhibited, as shown statistically, the

Table 1. The influence of drug affecting dopaminergic system on ethanol withdrawal audiogenic seizures in rats

Drugs	Dose mg/kg	Rats with seizures %
Amantadine ($n=11$)	25	54.6 $p<0.01$
Control ($n=10$)		90
Bromocriptine ($n=8$)	2.5	87.5
Bromocriptine ($n=8$)	5	87.5
Control ($n=8$)		75
Bromocriptine ($n=13$)	10	92.3
Control ($n=13$)		76.9
Sulpiride ($n=12$)	10	83.3
Control ($n=12$)		83.3
Metoclopramide ($n=13$)	10	76.9
Control ($n=13$)		76.9
Haloperidol ($n=12$)	0.5	91.7
Control ($n=12$)		83.3

appearance of audiogenic seizures in rats, during ethanol abstinence. Amantadine is a potent inhibitor of dopamine uptake by dopaminergic neurones and in large doses, it releases dopamine. The experiments of Blum et al. (3) prove that our results are right because L-DOPA as well as intracerebrally administered dopamine reduced seizures in mice during ethanol abstinence. These investigations made it obvious that the increase of the dopamine level in the central nervous system inhibited ethanol withdrawal seizures.

The results of some biochemical research both prove and suggest additionally that long-term administration of ethanol may evoke hypofunction of dopaminergic neurons. Fadda et al. (6) found that a single or long-term administration of ethanol releases dopamine for neuronal stores. However, the release of dopamine resulting from a single administration of ethanol is accompanied by the increase of dopamine synthesis. This phenomenon does not occur with long-term treatment by ethanol. It was found by Tabakoff and Hoffman (13) that dopamine synthesis is lowered when there are symptoms of ethanol abstinence. Darden and Hunt (5), too, reached the results showing that the release of dopamine from the striatum is considerably reduced during ethanol abstinence.

Our findings while administering bromocriptine do not correspond to the earlier research. This substance stimulates the central dopamine receptors, the fact which is used in treatment of some endocrinological and neurological diseases (10). In none of the doses used (2.5; 5 and 10 mg/kg) did bromocriptine have its statistically essential effect on the appearance of audiogenic seizures.

Other results were obtained by Trzaskowska et al. (14). Bromocriptine in a dose 2.5 mg/kg decreased ethanol withdrawal seizures in rats. Audiogenic seizures in these investigations were assessed according to Job et al. (9). Borg and Weinhold (4) examined the influence of bromocriptine on the symtoms of ethanol abstinence in people. This substance strongly reduced such psychiatric manifestations as fear, anxiety and depression and much less affected the remaining symptoms of abstinence, that is tremors, sweating, nausea. The research did not reveal how bromocriptine influenced seizures appearing in people dependent on ethanol. Our other investigations prove that bromocriptine in a dose of 2.5 mg/kg reduces ethanol withdrawal head twitches in rats. Head twitches are the earlier and weaker mark of ethanol abstinence than the audiogenic seizures.

Metoclopramide and sulpiride, being selective antagonists of dopaminergic receptors D-2, did not enhance audiogenic seizures. Haloperidol, too, did not have any essential influence on the appearance of that symptom. This neuroleptic, similar to spiroperidol, is a derivative of butyrophenon. It was proved that the neuroleptics of this group are strongly related to dopaminergic receptors D-2 (11, 12). It seems then that the substances which block the dopaminergic receptors D-2 (metoclopramide, sulpiride and haloperidol) do not have any influence on ethanol withdrawal audiogenic seizures in rats. The observations are not in agreement with the results obtained in mice because it has been proved that haloperidol intensified ethanol withdrawal seizures (2).

Reduction of the amount of ethanol withdrawal audiogenic seizures due to amantadine implies that the appearance of this symptom may be, to some extent, related to the lowered activity of the dopaminergic system. However, the results obtained after administration of bromocriptine do not prove it. This substance has, yet a different mechanism of activating neurons of the dopaminergic system than amantadine. In our opinion the lack of bromocriptine influence on ethanol withdrawal audiogenic seizures do not exclude a possibility of observing hypofunction of dopaminergic neurons after the long-term administration of ethanol. Such a system may participate to a greater extent in the appearance of other symptoms of ethanol abstinence than audiogenic seizures. The lack of the influence of the antagonists of the dopaminergic system on the appearance of ethanol withdrawal audiogenic seizures is not in disagreement with our conclusion.

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STRESZCZENIE

Szczury samce szczepu Wistar uzależniano przez dożołądkowe podawanie 40% (v/v) wodnego roztworu etanolu, który podawano co 8 godzin przez kolejnych 5 dni. Ocenianym objawem abstynencji były drgawki audiogenne, wywoływane przy użyciu dzwonka elektrycznego. Bromokryptyna w dawce 2,5; 5 i 10 mg/kg nieznacznie nasilała drgawki audiogenne. Istotne statystycznie hamowanie drgawek audiogennych obserwowano po podaniu amantadyny w dawce 25 mg/kg. Metoklopramid (10 mg/kg) i sulpiryd (10 mg/kg) pozostawały bez wpływu na drgawki audiogenne u uzależnionych szczurów. Również haloperidol (0,5 mg/kg) nie miał istotnego wpływu na powstawanie tego objawu abstynencji.

Zmniejszenie ilości drgawek audiogennych u uzależnionych szczurów przez amantadynę sugeruje, że powstawanie tego objawu abstynencji może być w pewnym stopniu związane ze zmniejszoną aktywnością układu dopaminergicznego. Nie potwierdzają tego wyniki uzyskane po podaniu bromokryptyny. Naszym zdaniem, brak wpływu bromokryptyny na powstawanie drgawek audiogennych u uzależnionych szczurów nie wyklucza możliwości wystąpienia hipofunkcji neuronów dopaminergicznych po przewlekłym podawaniu etanolu.