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Inhibitory Influence of Dihydroergosine on the Aggressiveness of Rats and Mice

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MANEV, H., D. PERIČIĆ AND D. MÜCK-ŠELER. *Inhibitory influence of dihydroergosine on the aggressiveness of rats and mice.* PHARMACOL BIOCHEM BEHAV 32(1) 111–115, 1989.—Fifty mg/kg of ergot alkaloid dihydroergosine (DHESN) inhibited the mouse-killing behavior of isolated male rats, while 10 mg/kg did not. This effect of DHESN (50 mg/kg) lasted for 24 hr. When an additional injection of DHESN (50 mg/kg) was given to mouse-killer rats 6 days following the first, the mouse-killing behavior was again inhibited. The effect of the second drug injection also persisted for 24 hr and was accompanied by an increased concentration of 5-HT in the raphe nuclei and hypothalamus and by a decreased concentration of GABA in the olfactory bulbs. DHESN also inhibited aggressiveness in isolated mice. Two hr following the administration of 10 mg/kg DHESN the fighting was inhibited in 46% of pairs tested, while 50 mg/kg abolished it completely. The effect of 50 mg/kg lasted 24 hr. These results, showing the antiaggressive effects of DHESN, support our previous suggestion that DHESN might presumably be a new antidepressant, and suggest that besides the serotoninergic, the GABA-ergic system might also be involved in the modification of behavior induced by this drug.

Dihydroergosine Mouse-killer rats Aggressive mice 5-HT GABA Antidepressant properties

DIHYDROERGOSINE (DHESN) is one of the naturally occurring ergot alkaloids. Recently, some neuropharmacological properties of this substance have been discovered. DHESN has been shown to antagonize the effects of noradrenaline on the postsynaptic and extrajunctional alpha-adrenoceptors (25), to decrease the turnover of serotonin (5HT) in the whole brain, to lower the concentration and uptake of 5-HT in platelets (23) and to potentiate the 5-HT syndrome in the rat (21,23). Besides having a pronounced hypotensive effect (27) according to our previous investigations showing the effectiveness of DHESN in the behavior despair test (19,23), this drug might also be considered as a potential antidepressant agent.

Social isolation of rats and mice induces the development of various types of aggressiveness. In rats this may be expressed as mouse-killing behavior, and in mice as vicious fighting behavior (28). Antidepressants have been shown to suppress these types of aggressive behavior (5, 26, 28). Moreover, it has been stressed that their ability to inhibit the mouse-killing behavior may occur in doses below the neurotoxic (26). Thus, the inhibition of mouse-killing behavior may, in spite of some pitfalls of this technique, be considered as predictive for antidepressant activity (30).

There is sustained evidence for the involvement of gamma-aminobutyric acid (GABA) and 5-HT neurotransmitter systems in the genesis of both of the above mentioned aggressive behaviors. Moreover, the olfactory bulbs have been suggested as the site of action of GABA, while the raphe nuclei and hypothalamus have been proposed as the site of action of 5-HT (18). Although the influence of GABA in the genesis of mouse-killing behavior is still not quite clear (6,18), there is good agreement on the inhibitory influence of brain 5-HT on this type of behavior (7,16).

The aim of this study was to investigate the influence of this drug on the mouse-killing behavior in isolated rats and on the isolation-induced aggressiveness in mice. At the same time we intended to investigate the influences of DHESN on the concentration of GABA in the olfactory bulbs, and on the concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the raphe nuclei and hypothalamus of mouse-killer rats.

METHOD

Male Wistar rats and male BALB/c and CBA mice from our institute were used. The rats and albino BALB/c mice were caged individually from approximately 30 days of age, and CBA mice (3 months old) were caged individually 2 weeks prior to experiment. The animals were kept at a constant temperature (22°C) and in a light cycle of 11 hr light/13

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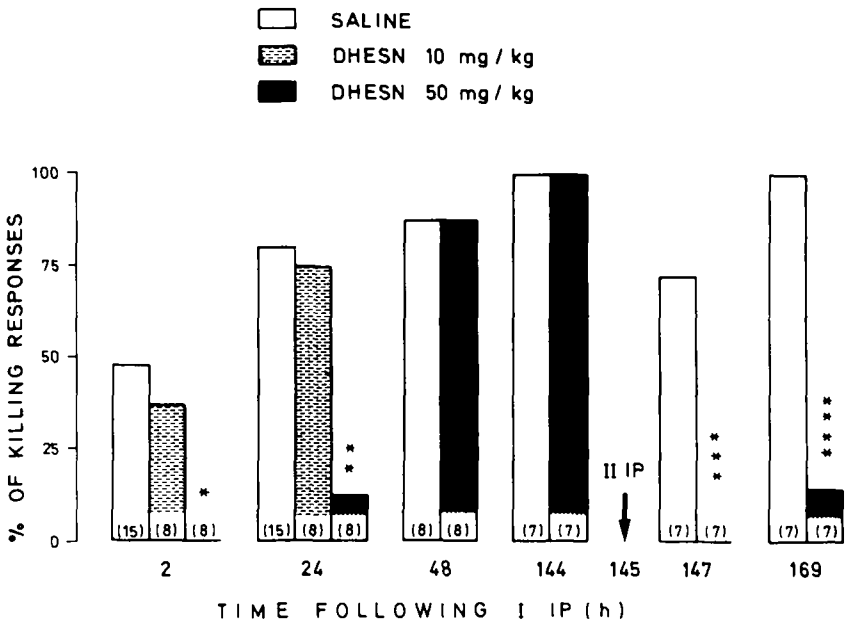

FIG. 1. Effect of dihydroergosine (DHESN) on mouse-killing behavior induced in rats by social isolation. Before IP injection of either saline or DHESN (10 and 50 mg/kg) rats were tested for mouse-killing behavior as described in the Method section. Three experimental groups were selected, each showing 100% killing response. Killing response was retested 2 and 24 hr, and for saline- and DHESN- (50 mg/kg) treated groups 48, 144, 147 and 169 hr following the IP injection. Second IP injection (saline or DHESN, 50 mg/kg) was administered 145 hr following the first one. One hr following the last testing, the animals were sacrificed and brains submitted to biochemical analysis. The results are expressed as the percentage of the animals which killed the mouse. Numbers in the bars represent the number of animals in the group. $*p<0.05$, $**p<0.025$, $***p<0.01$, $****p<0.005$ when compared with the corresponding saline-treated control (Fisher's test).

hr darkness (light on at 7.00). They were given food and water ad lib. All experiments were carried out between 8.00 and 13.00 hr.

Dihydroergosine methane sulphonate (DHESN, Lek, Ljubljana, Yugoslavia) was dissolved in distilled water (doses expressed as salt) and injected intraperitoneally (IP). Control animals were injected IP with saline (1 mg/100 g of body weight).

Mouse-killing behavior was induced in male rats by 7 weeks of isolation. The rats were caged individually in Plexiglas cages (37×27×15 cm). Visual contact with other animals was made impossible and the cages were cleaned once a week. During the seventh week of isolation they were tested 3 times for the presence of mouse-killing behavior by placing the adult male CBA mouse for 60 min in their home cage. Only the regular killers (approximately 60% of the original colony) were used for drug experiments.

BALB/c mice were kept singly in Plexiglas cages (18×13×12 cm) for 4 weeks. The cages were cleaned once a week. After 4 weeks of isolation the mice were tested for aggressiveness by placing the naive CBA mice in their home cage. If an attack ensued within the first 10 min the pair was selected for further study. Since that time CBA mice were also isolated. Each pair was retested twice, and the pairs were kept constant during the experiments. The experiments were performed after the mice, BALB/c and CBA, had been isolated for 6 and 2 weeks respectively. Both mice in the pair (BALB/c and CBA) received the same, drug or saline, IP injection. The pairs were tested for aggressiveness during a 10-min test period. They were separated after the first attacking or biting response, or at the end of a 10-min testing period.

In some experiments the olfactory bulbs, raphe nuclei and hypothalami of rats were taken for biochemical analyses. For this purpose the animals were killed by exposing their heads to a focussed beam of microwave irradiation for 5.0 sec (10). After sacrifice the brains were rapidly removed from the skulls and placed in a tissue slicer over an ice cold plate. A tissue slicer with a brain shaped depression 22 mm long, with slits at 1.5 mm intervals, was used to obtain the region of hypothalamus and raphe nuclei, which were dissected according to the stereotaxic atlas of the rat brain (15). The tissue of these two brain regions was analysed for 5-HT and 5-HIAA levels by a slight modification of the method of Curzon and Green (3). The olfactory bulbs were also taken and analysed for the concentration of GABA by a modification (24) of the enzymatic fluorimetric method (13). Protein concentrations were determined in 10 μl of the homogenates according to Lowry *et al.* (17).

Statistical analysis of the results was performed either by Fisher's test (9) or two-tailed Student's t-test. The criterion for significance was $p<0.05$.

RESULTS

Although only the determined mouse-killers were selected for the study, 2 hr after the IP injection of saline the killing response decreased from 100% before IP (data not

TABLE 1

EFFECT OF DIHYDROERGOSINE (DHESN) ON THE CONCENTRATION OF 5-HT AND 5-HIAA IN THE RAPHE NUCLEI AND HYPOTHALAMI OF MOUSE-KILLER RATS

	(nmol/g tissue)		
	5-HT	5-HIAA	(n)
Raphe nuclei			
Saline	6.96 ± 0.19	5.41 ± 0.20	(7)
DHESN	8.08 ± 0.25†	5.83 ± 0.17	(6)
Hypothalamus			
Saline	6.14 ± 0.47	6.45 ± 0.32	(7)
DHESN (50/kg)	7.28 ± 0.17*	6.68 ± 0.29	(6)

The experimental procedure was the same as described for Fig. 1. Animals were sacrificed by exposing their heads to microwave irradiation 60 min after the last testing (Fig. 1: 169 hr following I IP). Results are the mean ± SEM of (n) animals in the group.

*$p<0.05$, †$p<0.01$ when compared with the corresponding saline-treated group (Student's t-test).

TABLE 2

EFFECT OF DIHYDROERGOSINE (DHESN) ON THE CONCENTRATION OF GABA IN THE OLFACTORY BULBS OF MOUSE-KILLER RATS

Treatment	GABA (nmol/mg protein)	(n)
Saline	24.62 ± 1.28	(7)
DHESN (50 mg/kg)	18.27 ± 0.69*	(6)

The experimental procedure was the same as described for Fig. 1. Animals were sacrificed by exposing their heads to microwave irradiation 60 min following the last testing (Fig. 1: 169 hr following I IP). Results are the mean ± SEM of (n) animals in the group. *$p<0.01$ when compared with the saline-treated group (Student's t-test).

shown) to 47% after IP injection (Fig. 1). When compared with the control saline- treated animals the administration of DHESN in a dose of 10 mg/kg did not affect the killing response of mouse-killer rats neither 2, nor 24 hr after the administration. Fifty mg/kg of DHESN given 2 hr prior to testing completely abolished the mouse-killing behavior. Although the rats did watch the mouse, they were mostly peaceful. Only one out of eight rats attacked, but did not kill the mouse. Antimuricide action of DHESN was still very pronounced 24 hr following drug administration, however, it ceased after 48 hr (Fig. 1). Six days (144 hr) following either saline or 50 mg/kg of DHESN the killing response was again 100% in both groups. Two hr after the second IP injection (saline or 50 mg/kg DHESN), which was given 6 days after the first one, the killing response was again completely abolished in the DHESN-treated group, and this inhibitory effect of DHESN was still very pronounced one day later (Fig. 1).

One hr after the last testing of mouse-killing behavior, which was performed 169 hr following the first, i.e., 24 hr following the second IP injection of either saline or 50 mg/kg DHESN (Fig. 1) the rats were sacrificed and several brain regions were submitted to biochemical analyses. As shown in Table 1, the concentration of 5-HT increased in the raphe nuclei and in the hypothalamus, while the concentration of 5-HIAA was unchanged. When the 5-HT/5-HIAA ratio was calculated, a tendency to its enhancement was observed in both the brain regions, however the difference was not statistically significant (data not shown). DHESN treatment also decreased the concentration of GABA in the olfactory bulbs of DHESN-suppressed mouse-killers (Table 2).

The administration of DHESN (10 mg/kg) to aggressive isolated male mice (only the proved fighting pairs were selected for the study) 2 hr prior to testing, inhibited aggressiveness in 46% of the pairs tested, while 50 mg/kg abolished it completely (Fig. 2). After 24 hr the effect of 50 mg/kg was still present, ceasing 48 hr following drug administration (Fig. 2).

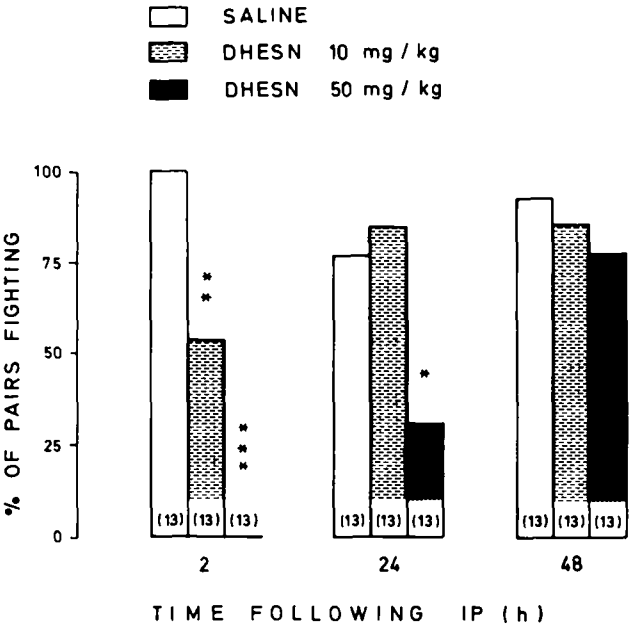

FIG. 2. Effect of dihydroergosine (DHESN) on the aggressiveness induced in mice by social isolation. Saline or DHESN were administered IP and the aggressiveness was tested 2, 24, and 48 hr later. Aggressiveness is expressed as the percent of pairs (BALB/c-CBA) which engaged in fighting during the 10-min testing period. Numbers in the bars denote the number of pairs tested. *$p<0.025$, **$p<0.01$, ***$p<0.005$ when compared with the saline-treated control (Fisher's test).

DISCUSSION

The present results show that the potential antidepressant DHESN (19, 21–23) inhibits the mouse-killing behavior in socially-deprived male rats. The inhibition of this type of aggressive behavior by a particular drug may be considered as predictive for its antidepressant activity, although the specificity of the mouse-killing behavior as a model of depression has been disputed (30). Namely, many drugs are able to inhibit the mouse-killing behavior, but antidepressants produce this effect in doses significantly lower than those classified as neurotoxic (26). In our study, the inhibitory influence of DHESN on mouse-killing behavior was observed with 50 mg/kg, a dose which does not change the rotarod performance of the animals (Manev and Peričić,

unpublished) and decreases immobility in the forced swim test (19).

The antimuricide effect of DHESN (50 mg/kg) was still present 24 hr after drug administration. Pharmacokinetic studies have shown that the biological half-life of DHESN in rat is 13.6 hr (20). Hence, the possibility remains that DHESN might have one or more active metabolites. However, a slow dissociation of this drug from the binding sites, as already described for a closely related ergopeptine, dihydroergotamine (11), appears to us to be a more suitable explanation for the prolonged action of DHESN. A second injection of DHESN 50 mg/kg, given 6 days after the first one, elicited again the inhibition of mouse-killing behavior, which was also very pronounced 24 hr later (Fig. 1). At the same time, i.e., 24 hr after the second administration of DHESN, we found pronounced changes in the concentration of 5-HT in the raphe nuclei and hypothalami of DHESN-treated mouse-killers, and in the concentration of GABA in the olfactory bulbs.

The inhibitory influence of 5-HT on the mouse-killing behavior has been well documented (7, 16, 18, 28, 31). Corresponding with these findings, the ability of DHESN to potentiate the 5-HT syndrome (21,23), i.e., to stimulate 5-HT_1 receptors (22), might explain our present findings of the suppressed mouse-killing behavior in DHESN-treated mouse-killers. However, the fact that stimulation of 5-HT_1 receptors lasted much longer (144 hr) than the suppression of mouse-killing behavior (24 hr) appears to disagree with this hypothesis. In addition, Peričić *et al.* (23) have reported that the acute administration of DHESN 50 mg/kg decreases the turnover of 5-HT in the whole rat brain. Our present data on the 5-HT and 5-HIAA levels in the specific brain regions differ from those mentioned previously, particularly in the method used. Namely, in the present study the mouse-killer rats were used instead of the naive, and the animals were sacrificed 24 hr, and not 1 hr, after drug administration. Nevertheless, the present findings of the increased 5-HT levels accompanied by uneffected 5-HIAA levels and 5-HT/5-HIAA ratios in DHESN-treated mouse-killers might be interpreted as a reflection of a diminished release of serotonin due to a sustained 5-HT_1 receptor stimulation. However, two other properties of this drug should be mentioned. Namely, besides stimulating 5-HT_1 receptors, DHESN blocks 5-HT_2 receptors as evidenced by inhibition of the head-twitch behavior in mice and rats (22) and alpha-adrenoceptors (25) whose role in the development of muricidal behavior has also been emphasized (14).

Another neurotransmitter system that should be considered in relation to DHESN-induced suppression of muricide is the GABA system. DHESN decreased the concentration of GABA in the olfactory bulbs of mouse-killer rats in which the killing response was inhibited by DHESN administration (Table 2). It has been reported that mouse-killer rats have lower values of GABA in the olfactory bulbs, and that the impairment of GABA-mediated inhibition by infusion into the olfactory bulbs of GABA-blocking drugs is able to elicit the mouse killing behavior in nonkiller rats (18). Our present findings on the influence of DHESN on the mouse-killing behavior and GABA levels in the olfactory bulbs appear to disagree with these literature data. However, it has been reported that some types of aggressive behavior may be accompanied by elevated GABA levels in the olfactory bulbs (12). Having in mind that the concentration of GABA per se does not necessarily reflect the activity of the GABA system, and that a decrease of GABA levels might even be the reflection of an enhanced GABA release, one may conclude that further studies are needed to clarify the involvement of the GABA system in DHESN-induced inhibition of mouse-killing behavior in rats.

The present study also shows that DHESN was able to inhibit, dose-dependently, the aggressiveness induced in mice by social isolation. Besides benzodiazepines that have a more or less specific effect on aggressive response in isolated animals, antidepressants also may inhibit this kind of aggressiveness in mice (5, 8, 26). Since isolation-induced aggressiveness might be connected with the impaired serotoninergic (28), but also GABA-ergic (1,24) system, our present findings indicating antiaggressive properties of DHESN may presumably be explained by action of this drug on these neurotransmitter systems, although as in the case of muricide suppression the effects of this drug on alpha-adrenoceptors (25) should not be neglected.

In conclusion, the potential antidepressant agent DHESN has been shown to possess the pronounced antiaggressive properties in two models of isolation-induced aggressiveness: the mouse-killing behavior in rats and the fighting behavior in mice. The DHESN-induced inhibition of mouse-killing behavior was accompanied by the changes in the GABA and 5-HT neurotransmitter system. Although further studies are required to elucidate the connection between these biochemical and behavioral effects of DHESN, the present findings are in corroboration with already suggested antidepressant properties of this drug.

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