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MUSCIMOL CHANGES HYPOXIA-INDUCED IMPAIRMENT OF BEHAVIOR IN RATS

Robert Oksztel, Halina Car, Konstanty Wiśniewski[#]

Department of Pharmacology, Medical Academy, Mickiewicza 2c, PL 15-222 Białystok, Poland

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Muscimol, a selective agonist of GABA-A receptors, causes changes in behavioral activity. Hypoxia interferes with the GABAergic system and with the functions of GABA-A receptors. We used muscimol in Wistar rats to estimate its influence on locomotor activity in the open field test as well as on the processes of consolidation and retrieval, evaluated in the test of passive conditioned reflexes. Anxiolytic activity was examined in the elevated "plus" maze in physiological state and after hypoxia-induced amnesia. Following intraperitoneal administration of muscimol (1 mg/kg, *ip*), the animals showed a decrease in motility, in retrieval of skill reflexes and in a number of entries into open and closed arms in the elevated plus "maze". In animals exposed to hypoxia, we observed reduced motility in the open field, inhibition of retrieval and consolidation of passive conditioned reflexes, shortened time of sojourn in open arms and decreased number of entries into open and closed arms. In the group of animals which underwent hypoxia and then received muscimol, we observed no effect of hypoxia on muscimol activity in the open field test, except rearing when muscimol action was significantly reduced. Muscimol improved consolidation but not retrieval in comparison with the hypoxic saline-treated group of rats. In the elevated "plus" maze test, treatment of rats with muscimol after hypoxia significantly prolonged the time spent in open arms and increased the number of entries into open arms, while shortened the time spent in closed arms. In conclusion, muscimol in hypoxia-exposed group of rats exerted beneficial effect on consolidation in passive avoidance situation and exerted anxiolytic activity. Changes in the activity of muscimol under hypoxia may have significant clinical implications.

Key words: *muscimol, hypoxia, behavior, rats*

[#] *correspondence*

INTRODUCTION

In the mammalian central nervous system, γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter. Conformationally restricted analogues of GABA have been used to help identify three major GABA receptors, termed GABA-A, GABA-B and GABA-C receptors [9]. GABA-A receptors are hetero-oligomeric Cl^- channels that are selectively activated by muscimol, blocked by the alkaloid bicuculline and modulated by steroids, barbiturates and benzodiazepines [9, 19]. The stimulation of GABA-A receptors by endogenous agonist, GABA, induces hyperpolarization through the increased inflow of Cl^- to cells, which electrophysiologically appears as a quick potential inhibiting stimulation (fast IPSP). Sharma and Kulkarni [41] demonstrated the role of this receptor in memory processes. GABA is released in different brain areas during learning of different tasks and after the induction of LTP [31]. The results further suggest a role of neurosteroids and GABA-A receptors in the modulation of emotional behavior and mood [40]. Modulation of this receptor activity by its agonist, muscimol, shows weakness of retrieval examined in behavioral tests with negative stimuli [16, 25, 41]. Observations of clinical patients in the state of anoxemia have indicated possible GABAergic mechanism of this pathology.

Hypoxia produces a series of changes in the central nervous system including disruption of synthesis, release and uptake of numerous neurotransmitters, e.g. an increase in extracellular glutamate, GABA and taurine concentration [15, 39]. High concentrations of excitatory amino acids (EAA) and free radicals released under cell-damaging conditions are neurotoxic and contribute to neuronal death during hypoxia [18]. Experimentally induced hypoxia interferes with the GABAergic system and with the functions of GABA-A receptors, intensifying GABA release in the central nervous system [29, 45], and enhancing density and expression of mRNA for receptor subunit [10, 22, 28, 36, 38]. The number of [^{3}H] muscimol binding sites was increased significantly following exposure to hypoxia. The receptor number tended to return to control values after 24 h. These results may represent an up-regulation of postsynaptically located GABA-A receptors corresponding to the impaired presynaptic activity under hypoxia [28]. Our previous study established that hypoxia (2% O_2, 98% N_2) impaired the consolidation and retention of the passive avoidance response [4–6]. Since there is evidence that experimental amnesia interferes with the functions of GABA-A receptors, we can also expect changes in behavioral effects mediated by this receptor.

The aim of the present investigations was to analyze behavioral effects of muscimol, an agonist of GABA-A receptors, under physiological conditions and after amnesia induced by hypoxia.

The advantageous effects of muscimol despite hypoxia-induced amnesia may have important clinical implications.

MATERIAL and METHODS

Subjects

Female Wistar rats of laboratory strain, weighing 160–180 g, were used. The animals were fed standard diet and housed in plastic cages (50 × 40 × 20 cm), 10 animals per cage, in an air-conditioned and temperature-controlled (22°C) room under a 12 h light/dark cycle beginning at 7.00 h. Food and water were freely available. All experiments were carried out in a quiet, diffusely lit room (25 W bulb, 2 m away from an animal, indirect light) between 8.00 h and 12.00 h.

Drugs

Muscimol (Tocris, Cookson Ltd., UK) was dissolved in 0.9% NaCl (pH 7.4) and administered intraperitoneally (*ip*) at a dose of 1 mg/kg [11, 33] 30 min before estimation of the animals' behavior in the open field and elevated "plus" maze tests, and immediately before hypoxia. In the passive avoidance test, it was given on the second or on the third day (see passive avoidance response training). Saline (0.9% NaCl) (Polfa, Poznań, Poland) was administered *ip* at a dose of 1 ml/kg at the same time as muscimol.

Amnesia induced by hypoxia

Hypoxia was induced by placing rats in a glass chamber flushed with a mixture of 2% O_2 in N_2 [2] till the respiratory arrest, after which they were immediately transferred to air. The hypoxia was induced after injection of muscimol, 30 min before open field and elevated "plus" maze tests; in the passive avoidance test immediately after completion of induction of passive avoidance on the second

day or on the third day. The drug tested was given immediately after completion of induction of passive avoidance on the second day (to determine it effect on consolidation) or 30 min before the retention test on the third day of experiments (to determine its effect on retrieval), respectively.

BEHAVIORAL TESTING

Locomotory and exploratory activity

The open field test was used to estimate the locomotor (crossings) and exploratory (rearings, bar approaches) activity of rats. The apparatus consisted of a square 100 × 100 cm white floor, which was divided by 8 lines into 25 equal squares, and surrounded by white walls, 47 cm high. Four plastic bars (designed as objects of possible interest), 20 cm high, were located in four different line crossings in the central area of the floor. A single rat was placed in the center of the floor and following 1 min of adaptation, crossings, rearings, and bar approaches were counted manually for 5 min. The crossings of the square were counted when the animal crossed the line with all four paws and the bar approaches were considered when the rat directed its head toward the bar approached and touched it with its nose.

Passive avoidance response training

The response was induced using the one-trial-learning method described previously [1]. The apparatus consisted of a 6 × 25 cm platform illuminated with a 25 W electric bulb, connected through a 6 × 6 cm opening with a dark compartment (40 × 40 × 40 cm). The floor of the cage was made of metal rods, 3 mm in diameter spaced at 1 cm. The investigation took advantage of the natural preference of rats to stay in dark compartments. The test lasted 3 days. On the first day, after 2 min of habituation in the dark compartment, the rats were placed on an illuminated platform, allowed to enter the dark compartment and then immediately removed. Two similar trials, at an interval of 2 min, were carried out on the second day. After the first trial, the rats were allowed to stay in the dark compartment for 10–15 s. At the end of the second trial, when a rat entered the dark compartment it received an inescapable footshock (0.25 mA, 3 s) delivered through the grill floor of the dark compartment (learning trial). The presence of the passive avoidance was checked 24 h later. The rats were placed on the illuminated platform once more and the latency to enter the dark compartment was measured, with the cutoff time of 300 s. To determine the effect of drug treatment on consolidation, according to the protocol proposed previously [23], muscimol was given on the second day immediately after completion of induction of passive avoidance. To determine effect on retrieval, it was administered on the third day 30 min before retention test.

Elevated „plus" maze test

The maze (constructed of gray colored wooden planks) consisted of two open arms, 50 cm (length) × 10 cm (width), and two enclosed arms, 50 cm (length) × 10 cm (width) × 40 cm (height), covered with a removable lid, so that the open or closed arms were opposite each other. The maze was elevated to a height of 50 cm from the floor. Ten minutes after the second injection, a naive rat was placed for 5 min in a pretest arena (60 × 60 × 35 cm, constructed from the same material) prior to exposure to the maze. This step allows the facilitation of exploratory behavior. The experimental procedure was similar to that described by Pellow et al. [34]. Immediately after the pretest exposure, the rats were placed in the center of the elevated „plus" maze facing one of the open arms. During the 5-min test period, the following measurements were taken: the number of entries into the open and closed arms and the time spent in the open and closed arms. An entry was defined as moving with all four feet into one arm. An increase in open arm entries and increase in time spent in the open arms is indicative of potential anxiolytic activity, as rats naturally prefer the closed arms.

Statistical analysis

Statistical significance of the results was computed by one-way analysis of variance (ANOVA) followed by Student's *t* and Newman-Keuls tests, except for passive avoidance behavior which was assessed with Mann-Whitney ranking test. F-rations, degrees of freedom and p-values are reported only for significant differences. In all comparisons between particular groups a probability of 0.05 or less was considered significant.

This work was approved by the Ethics Committee of Medical Academy in Białystok.

RESULTS

The effect of muscimol on locomotor and exploratory activity of control and hypoxia-exposed rats (Fig. 1)

Muscimol significantly decreased the number of crossed fields and rearings. Rats subjected to hypoxia exhibited a significant reduction in the number of crossed fields, rearings, and bar approaches. Rats pretreated with muscimol before exposure to hypoxia displayed significant reduction of rearings vs muscimol-treated control rats and vs saline-injected group of rats exposed to hypoxia, only.

The effect of muscimol on activity of control and hypoxia-exposed rats in the elevated "plus" maze (Fig. 2, 3)

Muscimol treatment of rats did not influence the time spent in closed and open arms, but reduced the number of entries into open and closed arms. Rats subjected to hypoxia showed significant shortening of the time spent in open arms and reduction of the number of entries into closed and open arms. Rats which received muscimol before exposure to hypoxia exhibited significant prolongation of the time spent in open arms, reduction of the time spent in closed arms and an increased number of entries into open arms in comparison with the hypoxic saline-treated and muscimol-treated control groups of rats.

The effect of muscimol on consolidation of passive avoidance in control and hypoxia-exposed rats (Tab. 1)

Muscimol did not influence consolidation of passive avoidance. The latency of entering the dark compartment was shortened in rats which underwent hypoxia, and this effect was conspicuously reversed by muscimol, vs hypoxic saline-treated group of rats.

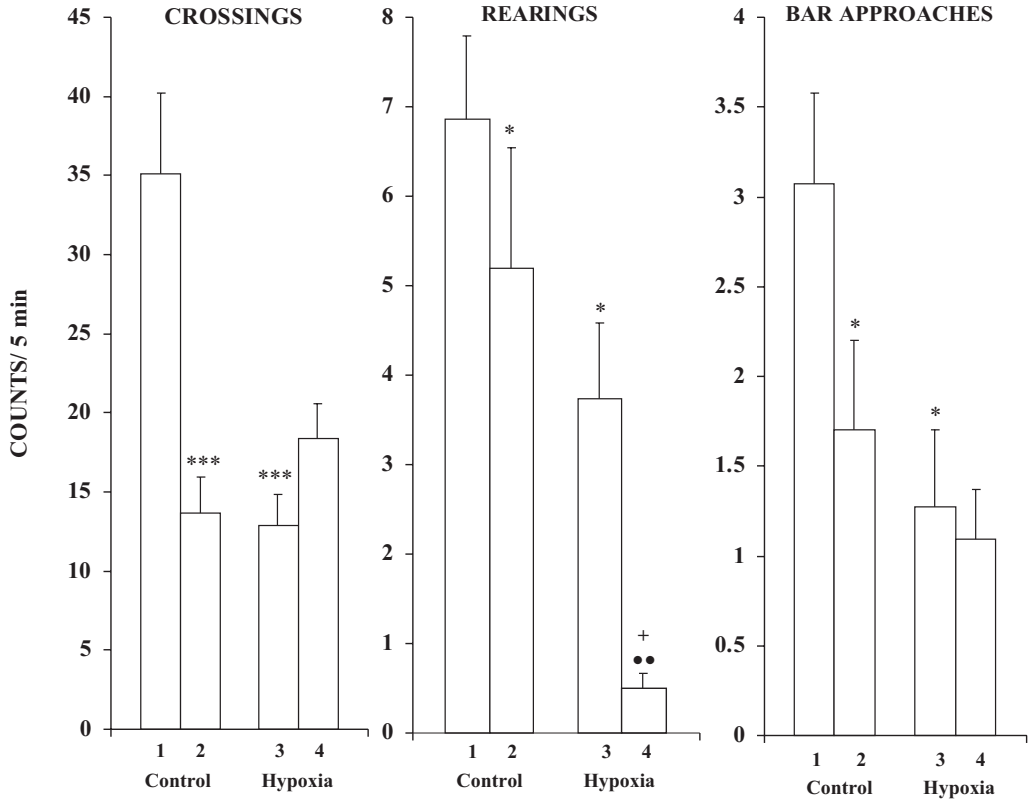

Fig. 1. The effect of muscimol on number of crossings, rearings bar approaches in the open field in control (groups 1–2), and hypoxia-exposed rats (group 3–4). Columns represent means ± SEM of the values obtained from 10–14 animals. 1) saline 1 ml/kg *ip*; 2) muscimol 1 mg/kg *ip*; 3) saline (1 ml/kg *ip*) and hypoxia; 4) muscimol (1 mg/kg *ip*) and hypoxia. Crossings $F(3.42) = 9.208$; *** $p(1\text{-}2,3) < 0.001$. Rearings $F(3.42) = 8.419$; * $p(1\text{-}2,3) < 0.05$; •• $p(2\text{-}4) < 0.02$; $^{+}$ $p(3\text{-}4) < 0.05$. Bar approaches $F(3.42) = 4.437$; * $p(1\text{-}2,3) < 0.05$; (ANOVA, Student's *t*-test, Newman-Keuls tests)

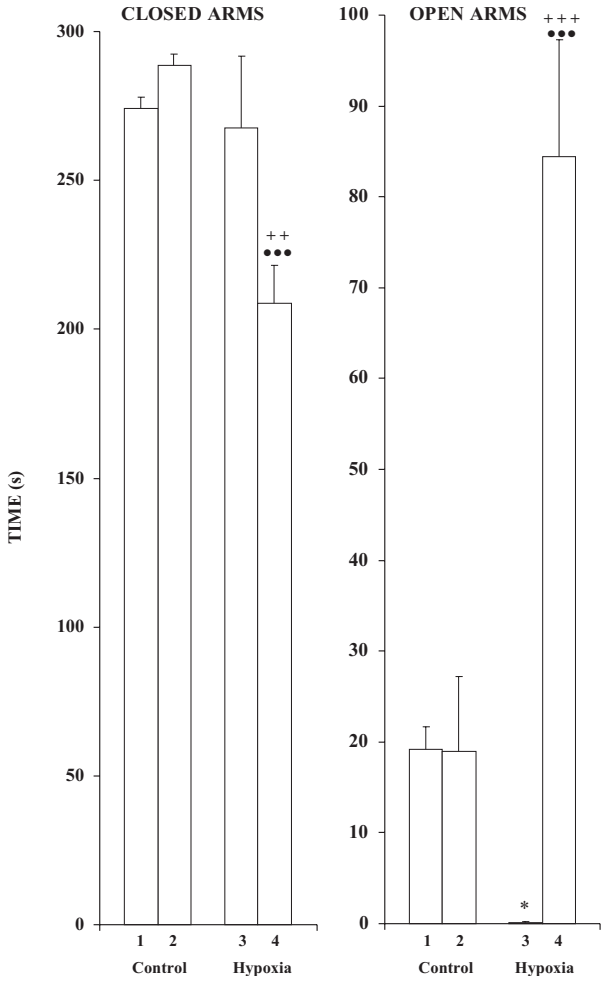

Fig. 2. The effects of saline (1), muscimol (2) and saline + hypoxia (3) and muscimol + hypoxia (4), on the time spent in closed and open arms in the elevated "plus" maze. For further details see text. Columns represent means ± SEM of the values obtained from 10–13 animals. Closed arms F(3.42) = 7.419; ••• p(2-4) < 0.001; ++ p(3-4) < 0.02. Open arms F(3.42) = 22.201 * p(1-3) < 0.05; ••• p(2-4) < 0.02; +++ p(3-4) < 0.001 (ANOVA, Student's *t*-test and Newman-Keuls tests)

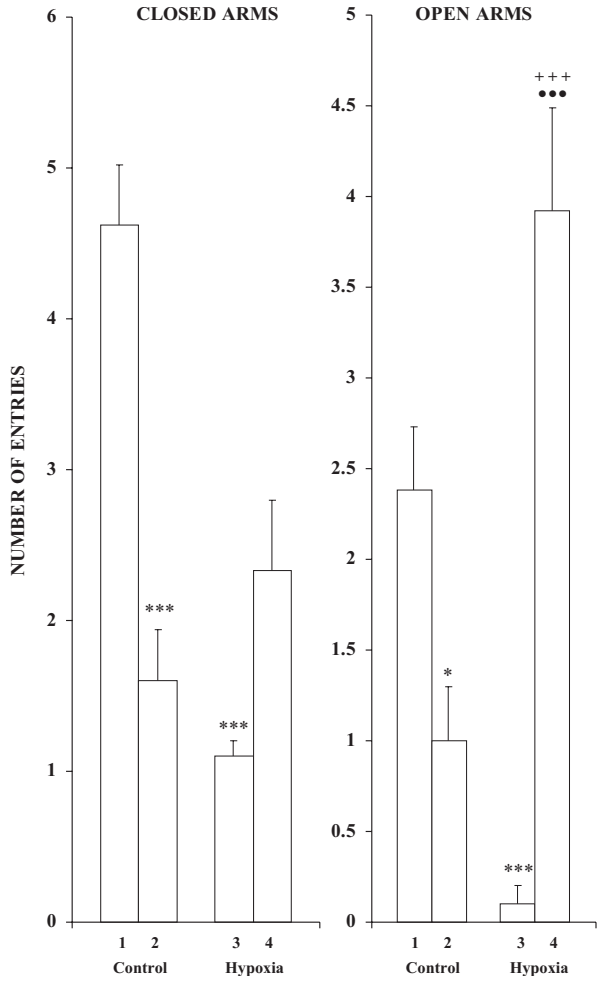

Fig. 3. The effect of saline (1), muscimol (2) and saline + hypoxia (3) and muscimol + hypoxia (4), on the number of entries into closed and open arms in the elevated "plus" maze. For further details see text. Columns represent means ± SEM of the values obtained from 10–13 animals. Closed arms: F(3.42) = 18.084; *** p(1-2,3) < 0.001. Open arms: F(3.42) = 17.752; *** p(1-3) < 0.001; * p(1-2) < 0.05; ••• p(2-4) < 0.001; +++ p(3-4) < 0.001 (ANOVA, Student's *t*-test and Newman-Keuls tests)

Table 1. The effects of muscimol on consolidation of passive avoidance in control and hypoxia-exposed rats

Treatment	n	Re-entry latency (s)
Saline	8	32.37 (10–62)
Muscimol	9	25.44 (12–46)
Saline + hypoxia	13	17.92 (10–29)**
Muscimol + hypoxia	13	34.84 (10–66)$^{++}$

The rats were treated *ip* with saline (1 ml/kg), muscimol (1 mg/kg) and were subjected to hypoxia. For further details see text. Median latencies are given, with the 25–75 percentiles in parenthesis. ** $p < 0.02$ as compared with saline-treated control group, $^{++}$ $p < 0.02$ as compared with saline-treated hypoxic group (Mann-Whitney test)

Table 2. The effects of muscimol on retrieval of passive avoidance in control and hypoxia-exposed rats

Treatment	n	Re-entry latency (s)
Saline	11	26.36 (17–44)
Muscimol	12	17.25 (10–27)**
Saline + hypoxia	13	15.84 (10–28)**
Muscimol + hypoxia	11	18.81 (10–37)

The rats were treated *ip* with saline (1 ml/kg), muscimol (1 mg/kg) and were subjected to hypoxia. For further details see text. Median latencies are given, with the 25–75 percentiles in parenthesis. ** $p < 0.01$ as compared with saline-treated control group (Mann-Whitney test)

The effect of muscimol on retrieval of passive avoidance in control and hypoxia-exposed rats (Tab. 2)

Muscimol significantly impaired retrieval of passive avoidance. Rats subjected to hypoxia had significantly shortened time spent on the platform. Rats pretreated with muscimol, and exposed to hypoxia exhibited no change in activity in this test vs. hypoxic saline-treated, and muscimol-injected control groups of rats.

DISCUSSION

In the present experiments, muscimol improved consolidation but not retrieval of passive avoidance in rats subjected to hypoxia, i.e. muscimol prevented consolidation of the hypoxia-induced deficit. Hypoxia significantly impaired these two processes. In physiological state, we observed no effect of muscimol on consolidation or impairment of retrieval in the passive avoidance situation.

Anatomical data evidence the presence of GABAergic neurons in the brain areas relevant to memory, such as the cortex, amygdala, septum, hippocampus, which together with the electrophysiological and biochemical changes induced by the learning experience suggests that the GABAergic neurons can critically modulate the electrical activity of these brain areas during the "multiple consolidation" process of memory storage [20]. The cognitive effects of the GABAergic agents are dose- and time-related. In accordance with our results, Castellano et al. [7, 8] and Wilensky et al. [46, 47] observed that activity of muscimol in the inhibitory avoidance learning paradigm is dose- and time-dependent. Immediate post-training infusion of muscimol had no effect on consolidation, but exerted an amnestic effect when given 90 or 180 min after training [17, 25, 48].

Functional injury of the hippocampus is one of particular effects of hypoxia. There is evidence that muscimol acts on the higher levels of the CNS, particularly on the hippocampus. Extracellular levels of glutamate and GABA increase during hypoxia. The elevated extracellular level of GABA may contribute to the maintenance of homeostasis in the hippocampus by impeding hyperexcitation. Drugs which potentiate the GABAergic systems also provide significant neuronal protection [1, 2]. Activation of GABA-A receptors in the hipocampus could be responsible for the inhibition of synaptic transmission during hypoxia [21].

The results of Ostrovskaia [32] also suggest that muscimol shows a pronounced antihypoxic effect. Cytoprotective mechanism of muscimol action in rabbit renal tubules subjected to hypoxia involves Ca^{+2} inhibition and subsequent Cl^{-} influx [44].

The mechanisms of hypoxia-induced impairment of memory retention reported in the literature are unclear. Hypoxia profoundly impairs retrieval of the passive avoidance response in rats and muscimol cannot prevent this effect.

In the present experiments, the stimulation of the GABA-A receptor by 1 mg/kg of muscimol given *ip* decreased number of crossings and bar approaches in the open field test. A similar reduction in locomotor activity after muscimol infusion into the ventral hippocampus was obtained by Bast et al. [3], Taira et al. [43], Dean et al. [13]. Hypoxia inhibited locomotor and exploratory activity, which suggests that it may mimic the inhibitory effect of spinal interneurons on motor activity. Significant inhibition of rearings was observed after muscimol administration in the hypoxia-exposed group of rats vs muscimol-injected control group of rats. In rats subjected to hypoxia and injected with muscimol the effects on crossings and bar approaches were similar to those obtained in rats which received muscimol and were not exposed to hypoxia. The results suggest a combined influence of muscimol and hypoxia on the dopaminergic systems and they may be disscused in terms of possible interaction of GABA systems with dopaminergic systems, whose activation has been shown to produce strain-dependent effects on memory processes as well [7].

The GABAergic system and especially the GABA-A receptor influence the dopaminergic system in different ways [24, 30]. The results obtained by Płaźnik et al. [35] indicate that muscimol has negative control of dopaminergic neurons in the nucleus accumbens septi, and, thus, plays an important role in behavior regulation in the rat. We applied muscimol at a dose of 1 mg/kg (*ip*) which probably reduces the activity of dopaminergic neurons.

On the other hand, the dopaminergic neurons in the whole brain are most sensitive to hypoxia. The results reported by Miwa et al. [26] suggest that hypoxia decreases dopamine (DA) biosynthesis and the DA turnover rate was remarkably lower through-

out the brain [14]. Hypoxia may also lower the activities of DA neurons [27] indirectly through the GABA interneurons which inhibit DA release [14].

The inhibition of locomotor and exploratory activity after hypoxia is a likely result of a considerable decrease in the activity of dopaminergic neurons. Locomotor and exploratory activity may affect the results obtained in the passive avoidance test although muscimol administered on the second day of this test does not interfere with motility in the consolidation process. The inhibition of locomotor activity induced by hypoxia and maintenance of this effect following muscimol administration did not impair retrieval in the passive avoidance situation in parallel groups of rats.

Since anxiety may influence the aversively motivated behavior, especially retrieval in passive avoidance situation, we examined the effect of muscimol on rats subjected to hypoxia in the elevated "plus" maze. The most significant anxiolytic-like activity of muscimol was obtained after hypoxia while it did not prevent anxiety under physiological conditions.

Muscimol decreased the number of entries into open and close arms in the elevated "plus" maze, and these effects corresponded to the inhibition of locomotor activity obtained in the open field test. Rats subjected to hypoxia exhibited anxiogenic activity; we observed a reduction in the time spent in open arms, and in the number of entries into open and closed arms. The latter effect may depend on the inhibition of locomotor activity in the open field test. Unexpected prolongation of the time spent in open arms, reduction in the time spent in closed arms, and increase in the number of entries into open arms were obtained in rats subjected to hypoxia and pretreated with muscimol. Locomotor activity had no influence on these effects in the elevated "plus" maze test. Therefore, evidence from the elevated "plus" maze test suggests that muscimol given before hypoxia exhibited anxiolytic activity. Manipulation of GABA-A receptor activity has been found to produce highly variable effects in animal models of anxiety [12]. Injection of muscimol into the dorsomedial hypothalamus caused a dose-dependent anxiolytic-like effect [42]. "Anxious" animals had significantly lower numbers of [^{3}H]-muscimol binding sites in the cerebral cortex as compared to "non-anxious" animals, and this indicates that behavioral anxiety in mice correlates with the decreased number of GABA-A receptor in the cerebral cortex [37].

In summary, hypoxia profoundly impaired the consolidation and retrieval processes in passive avoidance situation, reduced the locomotor and exploratory activity, and produced an anxiogenic effect in the elevated "plus" maze test. Muscimol, an agonist of GABA-A receptors, which itself inhibited the retrieval processes, decreased locomotor and exploratory activity, and had no effect in the "plus" maze test. In rats subjected to hypoxia, it exhibited a beneficial action on consolidation and anxiolytic-like effect.

In conclusion, muscimol administered *ip* at a low dose of 1 mg/kg can change hypoxia-induced consolidation deficit in the passive avoidance test and has anxiolytic-like effect. These results show that muscimol is effective following systemic administration in non-conditioned anxiety procedures, and seem to indicate potential therapeutic efficacy in certain states connected with anxiety, like hypoxia.

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