UDC 612.821.4

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FORCED SWIMMING STRESS-RELATED HYPOALGESIA: NONDEPENDENCE ON THE HISTAMINERGIC MECHANISMS

Received December 17, 2012.

In experiments on mice, we examined the effects of 3-min-long forced swimming sessions on indices characterizing the state of the nociceptive system. Thirty minutes after the forced swimming episode, significantly lower ($P < 0.05$) latencies of motor reactions in the hot plate and tail flick tests were observed. At the same time, times of licking the paw within the early and late phases of the formalin test, as well as numbers of writhings in the acetic acid test, became significantly ($P<0.05$) smaller. Thus, forced swimming-induced stress results in the development of a hypoalgesia state with respect to thermoinduced pain and chemoinduced somatic (formalin test) and visceral (acetic acid test) pain. Blockers of histamine H1 (cimetidine, 10 mg/kg) and H2 (chlorpheniramine, 15 mg/kg) receptors did not influence significantly ($P > 0.05$) the intensity of forced swimming-induced hypoalgesia in the tail flick and acetic acid-induced (writhing) tests. Thus, the histaminergic system is not significantly involved in the mechanisms of forced swimming-induced hypoalgesia.

Keywords: forced swimming stress, hypoalgesia, histaminergic system, cimetidine, chlorpheniramine.

INTRODUCTION

Stressful conditions have been found to be a natural stimulus capable of triggering pain suppression. A number of observations demonstrated that pain perception is altered during exposure to various stressors [1]; the respective phenomenon is known as stress-induced hypoalgesia (SIHA). Among earliest reports on SIHA, there are results published by Beecher [2] who found that soldiers severely wounded in a battle reported little pain and required less analgesic medication, compared with civilians undergoing similar surgery. SIHA appears to be elicited by a wide range of influences, including thermal challenges, rotation, electric shock, exercise, and swimming. Laboratory rodents, after being exposed to forced swimming, manifest a decrease in pain sensitivity [3]. Currently, information on the mechanism(s) responsible for SIHA is still insufficient. Despite the accumulating evidence that histamine turnover is altered under physiological stress [4], the level of involvement of histaminergic mechanisms in response to stress has not been estimated.

Our experiments were carried out to investigate the possible role of the histaminergic system in forced swimming stress-related hypoalgesia.

METHODS

Animals. Male mice (50-80 g) were used for the study. They were housed and bred in the preclinical animal house of the College of Medicine (University of Ibadan, Nigeria) under standard laboratory conditions (room temperature and 12-h light/dark cycle). The animals were fed with standard mouse cubes (Ladokun feeds, Ibadan, Nigeria) and provided with water *ad libitum*.

The animals were subjected to a forced swimming procedure for 3 min by placing them in a plastic cylinder (diameter 30 cm, height 50 cm) containing water at 30°C (depth of 20 cm).

Drugs and Chemicals. The following drugs were used: cimetidine (SmithKline and Beecham, GSK,

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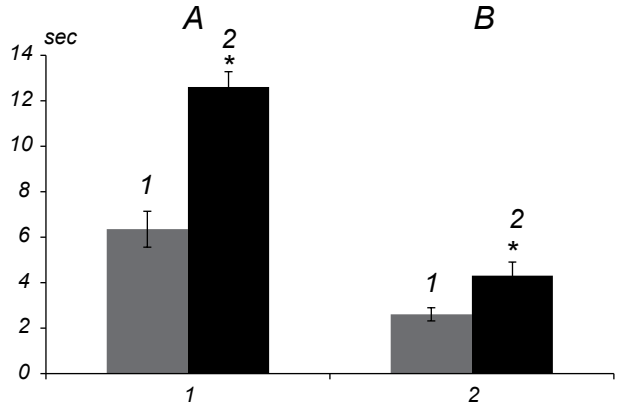
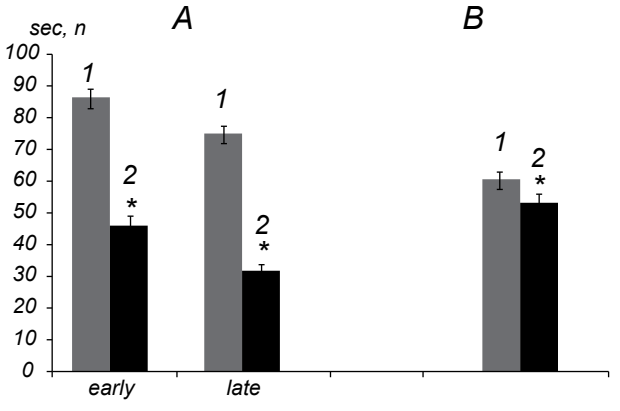


F i g. 1. Effects of swimming on hot plate and tail flick latencies (sec) in mice (A and B, respectively). Each value is the mean ± s.e.m.; n = 5. *$P < 0.05$ (non-swimming *vs* swimming, 1 and 2, respectively).

Р и с. 1. Вплив примусового плавання на латентні періоди моторних реакцій у тестах "гарячої пластинки" та "відсмикування хвоста" (с) у мишей (*A* та *B* відповідно).



F i g. 2. Effects of swimming on licking times in the formalin test (A) and number of writhings in the acetic acid test (B). Each value is the mean ± s.e.m.; n = 6. *$P < 0.05$ (non-swimming *vs* swimming, 1 and 2, respectively). Early and late are the respective phases of fam in the formaline test.

Р и с. 2. Вплив примусового плавання на тривалість облизування лапи у формаліновому тесті (*A*) та кількість викликаних болем корчів у тесті з ін'єкціями оцтової кислоти (*B*).

UK), chlorpheniramine maleate (Sigma-Aldrich, Germany), formaldehyde solution (Merck, Germany), and acetic acid (BDH, Great Britain).

Antinociceptive Assay. *Hot Plate Test.* The technique proposed by Eddy and Leimback [5] and modified by Ibironke et al. [6] was used. The mice were made to swim for 3 min. Thirty minutes later, they were placed on a hot plate (55 ± 2°C), and the time taken by the animal to jump off from the plate or lick its paws was taken as the hot plate/paw licking latency. No animal was allowed to stay on the hot plate for more than 60 sec to avoid excessive tissue damage. The mean latencies for each group were calculated. The numbers of animals (n) in the swimming and control groups were 6 each.

Tail Flick Test. The technique of D'Armour and Smith [7] was used. Briefly, the end (3 cm) of the tail was immersed in a water bath at 52°C after 3-min-long swimming. The time taken by the mouse to flick its tail out of hot water (tail flick latency) was measured.

Acetic Acid-Induced Writhing Test. The test was performed as previously described [8]. Each mouse was i.p. injected 30 min after swimming in water for 3 min with 0.2 ml of 3% acetic acid to induce characteristic writhings related to visceral pain. The number of writhings occurring between the 5th and 10th min of post-injection observation was measured.

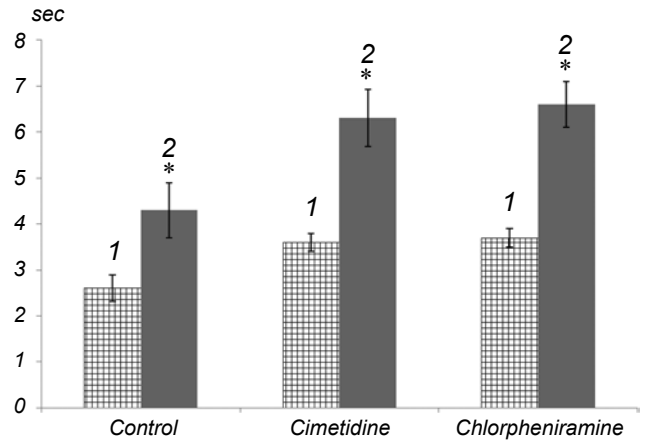
Formalin-Induced Paw Licking Test [9]. Thirty minutes after 3-min-long swimming, each animal was subcutaneously injected into the hind paw with 20 µl of 1% formalin, and the times taken to lick the paw within the first 5 min (acute pain phase) and for 10 min beginning from the 20th min post injection (tonic pain phase) were measured.

Pharmacological Modulation of Pain Reactions. In another set of experiments, the animals were pretreated with an H1 receptor antagonist, cimetidine (10 mg/kg), or an H2 receptor antagonist, chlorpherniramine (15 mg/kg), before being made to swim for 3 min. They were then subjected to the tail flick and acetic acid-induced writhing tests (n = 6), as described above.

RESULTS

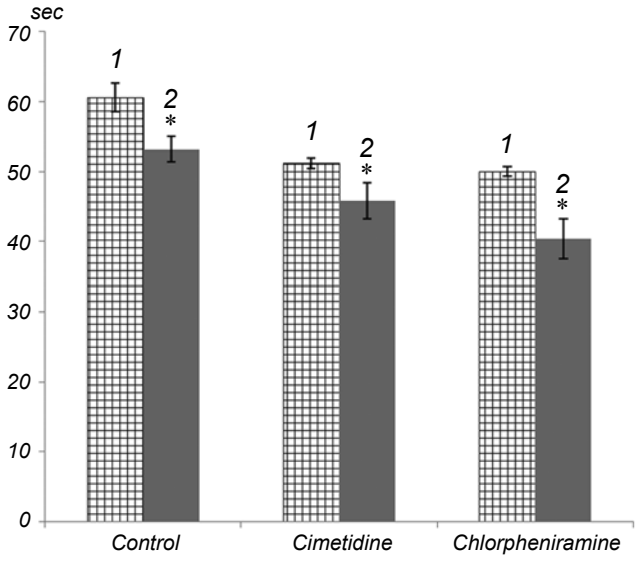
The mean latency of jumping off of rats from the hot plate in the respective test, when measured 30 min after episodes of forced swimming, was about two times longer than in the control; the normalized increment was 98.4%, $P < 0.01$ (Fig. 1A). Quite comparable differences were observed in the tail flick test; the tail flick latency after forced swimming was 65.4% longer than the corresponding value in the norm ($P < 0.05$, Fig. 1B). Thus, forced swimming resulted in a considerable drop in the sensitivity of the experimental animals to thermoinduced pain, i.e., in clear hypoalgesia.

Quite comparable changes in nociception were observed under conditions of the formalin test (chemoinduced somatic pain) and acetic acid



F i g. 3. Effects of cimetidine and chlorpheniramine on hypoalgesia induced by forced swimming stress in the tail flick test. Vertical scale) Tail flick latency, sec. Each value is the mean ± s.e.m.; n = 6. **P* < 0.05 (before *vs* after swimming, 1 and 2, respectively).

Р и с. 3. Вплив циметидіну та хлорфеніраміну на гіпоалгезію, що викликалася стресом, індукованим примусовим плаванням у тесті "відсмикування хвоста".



F i g. 4. Effects of cimetidine and chlorpheniramine on hypoalgesia induced by forced swimming stress in the acetic acid (writhing) test. Vertical scale) Number of writhes. Each value is the mean ± s.e.m., n = 6. **P* < 0.05 (before *vs* after swimming, 1 and 2, respectively).

Р и с. 4. Вплив циметидіну та хлорфеніраміну на гіпоалгезію, викликану примусовим плаванням, у тесті з ін'єкціями оцтової кислоти.

(writhing) test (chemoinduced visceral pain). The mean total duration of paw licking episodes within the early period of observation in the former test after 3-min-long forced swimming corresponded only to about half (53.2%) of the respective index in the absence of the mentioned influence. Forced swimming exerted an even somewhat stronger influence on the duration of pain licking within the late phase in the mentioned test (correlate of the intensity of tonic inflammatory pain); the above index was equal to 42.4% of the control value ($P < 0.05$ in both cases; Fig. 2A). The number of writhings in the acetic acid test also demonstrated a significant decrease (B).

Administration of both histamine receptor antagonists, cimetidine and chlorpheniramine, exerted certain modulatory effects on the indices characterizing the level of nociceptive reactions in the tests used. The tail flick latency in the corresponding test carried out in the case with no forced swimming became, after injections of these agents, about 35% longer than in the control. At the same time, administration of these drugs prior to swimming exerted no significant effects on normalized increases in the mentioned latency related to this influence. Under control conditions, the above increase was 63.6%; after cimetidine and chlorpheniramine injections, the respective values were 75.4 and 82.2%, respectively ($P > 0.05$ in both cases; Fig. 3).

In the writhing test, injections of the above antagonists also provided some basic hypoalgesic effects. The numbers of writhings in the two respective groups were about 15% smaller than in the sham-injected group. Normalized decreases in the above index in the cimetidine and chlorpheniramine groups, which were related to 3-min-long swimming, were 11.0 and 19.8% (Fig. 4), while the respective drop in the control group was 12.3%. In other words, injections of the mentioned agents exerted no significant effects on swimming-induced hypoalgesia with respect to visceral pain ($P > 0.05$ in both cases).

DISCUSSION

Our study clearly demonstrated the analgesic potential of forced swimming stress and its negligible dependence on the activity of histaminergic pathways. The characteristics of SIHA are dependent on the type and duration of stress, as well as the method by which hypoalgesia is assessed [10, 11]. In our experiments, forced swimming resulted in a hypoalgesic effect revealed by a significant ($P < 0.05$) prolongations of both hot plate and tail flick latencies (thermoinduced pain), as well as by significant ($P < 0.05$) reductions in

both the number of writhings in the acetic acid writhing test (visceral pain) and licking time in the formalin test. All these patterns remained nearly exactly the same despite prior administrations of both cimetidine and chlorpheniramine, indicating that SIHA in these cases is practically independent of the involvement of the histaminergic system.

Our observations on the effect of forced swimming stress on nociception are in agreement with earlier reports [12, 13]. However, our results contradict other observations [14, 15] where no analgesic effect was observed after swimming sessions. A few reasons could be adduced for this discrepancy. For example, the level of hypoalgesia was found to increase with age [16], mainly due to the development of the supraspinal descending inhibitory pathways. The cited authors who did not observe any analgesic effect probably used younger mice compared with animals used in our study.

Circadian rhythms also affect stress-induced hypoalgesia, as the pain sensitivity varies significantly with the time of day (being low in the morning and higher during daytime) [17]. Our experiments were carried out in relatively early hours of the day when the pain sensitivity is lower; hence the analgesic effects were easily observed compared with those carried out in the later part of the day, when analgesia could not be so easily observed because of increased pain sensitivity.

Despite the considerable amount of data regarding the analgesic effects of swimming-induced stress, the mechanism underlying these phenomena remains unclear. Various systems are supposed to be implicated in the mechanisms of SIHA, e.g., opioidergic [18] and endocannabinoid-related [19].

Thus, the results of our study have ruled out the possibility of significant involvement of histaminergic pathways in forced swimming-induced hypoalgesia. This conclusion is derived from the fact that pretreatment with H1 and H2 receptor antagonists failed to reverse or considerably modify the analgesic effect of forced swimming sessions.

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ГІПОАЛГЕЗІЯ, ПОВ'ЯЗАНА З ПРИМУСОВИМ ПЛАВАННЯМ: НЕЗАЛЕЖНІСТЬ ВІД ГІСТАМІНЕРГІЧНИХ МЕХАНІЗМІВ

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Р е з ю м е

В експериментах на мишах ми вивчали вплив сеансів примусового плавання тривалістю 3 хв на показники, що характеризують стан ноцицептивної системи. Через 30 хв після епізодів примусового плавання ми спостерігали істотно ($P < 0.05$) коротші латентні періоди моторних реакцій у тестах "гарячої пластинки" та "відсмикування хвоста". Водночас тривалість облизування лапи в межах ранньої та пізньої фаз формалінового тесту та кількість викликаних болем корчів у тесті з внутрішньоочеревинними ін'єкціями оцтової кислоти ставали істотно ($P < 0.05$) меншими. Отже, викликаний примусовим плаванням стрес призводить до розвитку стану гіпоалгезії щодо термоіндукованого болю, а також хемоіндукованого соматичного (формаліновий тест) та вісцерального (тест з використанням оцтової кислоти) болю. Блокатори гістамінових H1 (циметидін, 10 мг/кг) та H2 (хлорфенірамін, 15 мг/кг) рецепторів не впливали істотно ($P > 0.05$) на інтенсивність гіпоалгезії, викликаної примусовим плаванням, у тесті „відсмикування хвоста" та ацетатному тесті. Таким чином, гістамінергічна система не є залученою істотно в механізми індукованої примусовим плаванням гіпоалгезії.

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