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EFFECTS OF BETA-ADRENERGIC BLOCKADE ON DIABETES-INDUCED NEUROBEHAVIORAL ALTERATIONS IN MICE

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Neurobehavioral activities were estimated in three groups of male albino mice using the open field, elevated plus maze, light/dark board, and hole-board tests. The control group included intact animals, while alloxan-induced diabetes was evoked in the other two groups (single i.p. injection of 120 mg/kg alloxan). In the third group, a nonspecific beta-adrenoreceptor antagonist, propranolol, was i.p. injected (40 mg/kg) before the induction of diabetes. In diabetic mice, all neurobehavioral indices tested in the four above-mentioned tests were significantly ($P< 0.05$) smaller than those in the control group. The frequencies of rearings and grooming episodes in the open field, number of entries into the open arms and time spent in these arms in the elevated plus maze test, and number of head dips in the hole-board test demonstrated the most intense drops (more than twofold). Pretreatment with propranolol provided significant ($P < 0.05$) normalization of all neurobehavioral indices in diabetic mice; such normalization with respect to the locomotion intensity, frequency of grooming, time spent in the open arms, and both indices in the light/dark board was nearly complete. Thus, diabetes in the animal model used is accompanied by the development of the state of abnormally high anxiety. The activity of the beta-adrenergic system is noticeably involved in the formation of this state; pharmacological blocking of beta-adrenoreceptors provides significant anxiolytic effects.

Keywords: alloxan-induced diabetes, neurobehavioral indices, behavioral testing, anxiety, beta-adrenoreceptors, propranolol.

INTRODUCTION

As is generally known, *diabetes mellitus* affects the CNS and induces neurological dysfunctions and cerebrovascular impairments, even though the brain is not usually thought to be a special target for chronic diabetic complications [1]. Peripheral neuropathy was the primary neuroscience focus of diabetes research in the past; recently, however, chronic diabetes was found to affect significantly the CNS in several ways. In particular, it was found that diabetes causes cognitive dysfunction [2], dementia [3], deficits in the white matter microstructure [4], reduced hippocampal neurogenesis [5], and alterations in the gray matter density [6].

The adrenergic system is known to affect many neurobehavioral activities, including reconsolidation of appetitive learning [7], neurobehavioral and neurochemical alterations in STZ-induced diabetic rats [8], modulation of spatial memory, etc. [9]. At the same time, the role of the adrenergic system in diabetic alterations has attracted little attention. To enlarge the scope of study in this field, we focused on the beta-adrenergic modulation of CNS changes related to diabetes with emphasis on neurobehavioral phenomena and, specifically, on anxiety.

METHODS

Animals. Male Swiss Albino mice (25-35 g) obtained from the Pre-clinical animal house of the College of Medicine (University of Ibadan, Nigeria) were used in the study. They were kept at room temperature under standard laboratory conditions, with a 12-h light/dark cycle, and fed with mouse cubes (Ladokun feeds Nig Ltd., Ibadan) and water *ad libitum*.

Drugs and Chemicals. Propranolol hydrochloride and alloxan were obtained from Research Biomedicals

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Inc. (USA) and Sigma Aldrich (USA), respectively.

Induction of Diabetes. Diabetes was induced by a single dose of alloxan (120 mg/kg) injected intraperitoneally (i.p.) after a 24-h fast. Only mice with blood glucose levels above 185 mg/dl were considered diabetic and used for further tests.

Behavioral Assays.

Open Field Test. The standard technique of this test was used. The open field box looked like a rectangular arena with a hard floor, measuring 36×36×26 cm and made of white painted wood. The floor was divided by permanent red markings into 16 equal squares. Each mouse was introduced singly into one corner of the field, and the total locomotion index (number of units entered with all paws), rearing frequency (number of times the animal stood on its hindlimbs or with its four limbs against the walls of the observation box), and grooming frequency (number of body cleaning with paws, picking of the body and pubis with mouth, and face washing activities) within each 10-min-long interval were recorded.

After each of these assays, the arena was cleaned with 70% alcohol to eliminate olfactory bias and allowed to dry before introducing a fresh animal.

Elevated Plus Maze Test. The anxiety status of animals was assessed using the above-named test [10]. The elevated plus maze consisted of two open arms (30×5 cm) and two closed arms (30×5×15 cm) extended from a central platform (5×5 cm). The entire maze was elevated 40 cm above the floor. During the first 5 min of free exploration, the number of entries and the time spent in the open and closed arms were recorded. An entry was defined as the point where the animal placed all four paws onto the arm. The maze floor and walls were constructed from black and clear Plexiglas, respectively.

Light-Dark Exploration Test. The apparatus consisted of a Plexiglas box with two compartments (20×20 cm each), one of which was illuminated with white light, while the other remained dark. Each animal was placed at the junction of the light/dark areas, facing the illuminated compartment. The time spent in and the number of entries into the light and dark spaces were recorded for 5 min.

Hole–Board Test. The anxiety level was also evaluated in mice by using a hole-board apparatus (35×35×15 cm). Its walls were made from clear Plexiglas, and the arena was constructed from black Plexiglas and divided into 16 equal squares with 16 holes (diameters 3.5 cm). The equipment was elevated 56 cm above the floor. Each animal was placed on the central square of the arena, and the number of head dips was recorded during 5 min. An increase in the number of head dips signifies a positive anxiolytic-like effect [11].

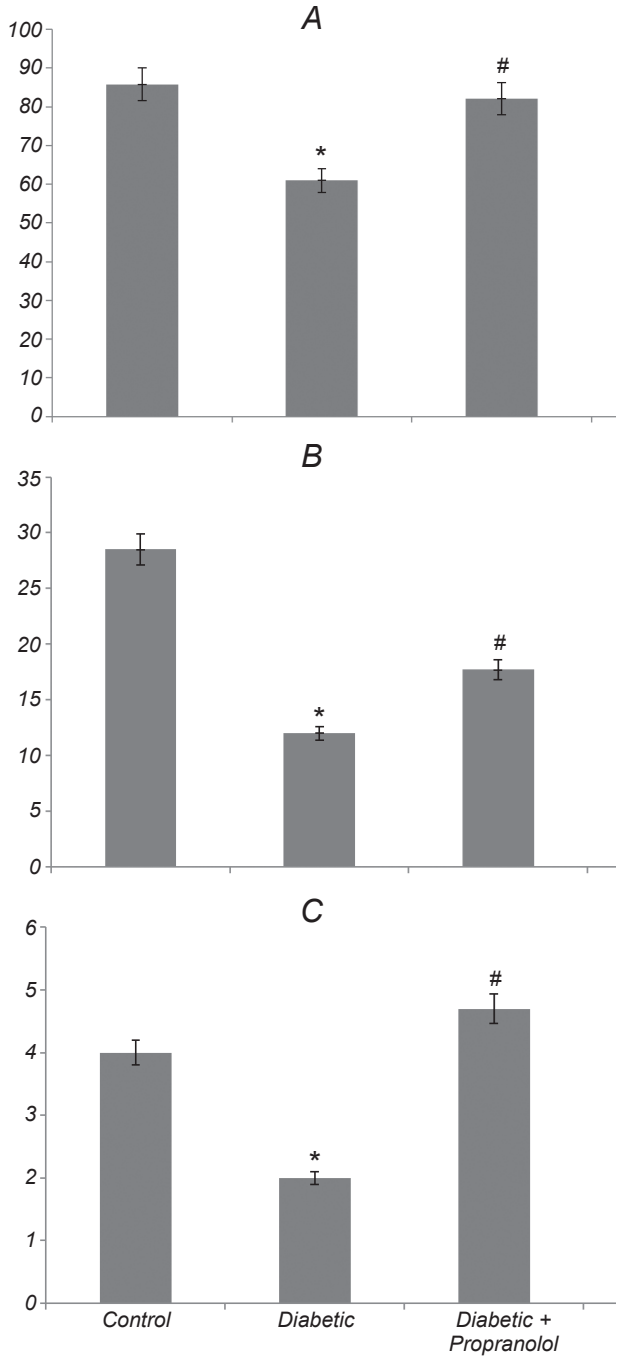
Statistical Analysis. Results were expressed as means ± s.e.m. The differences between behavioral data were analyzed using the Student's *t*-test and, at a value of $P < 0.05$, were regarded as significant.

RESULTS

Open Field Test. Mice with alloxan-induced diabetes demonstrated significant suppression of the locomotion phenomena in this test. In diabetic mice, the number of locomotion events within the observation period corresponded to only 71% of that in the control group ($P< 0.05$) (Fig. 1A). Even stronger suppression in these mice was found with respect to the indices of orientation/research behavior (rearings) and grooming episodes (42 and 49%, respectively, $P < 0.05$ in both cases) (Fig. 1B, C). Partial blocking of the beta-adrenergic system in diabetic mice due to pretreatment with propranolol led to significant increases ($P < 0.05$) in all above-mentioned indices. The intensity of locomotion in the respective group was practically fully normalized, and the frequency of grooming episodes even exceeded the control value. Only with respect to the number of rearings within the observation period was the recovery only partial; this index was equal to about 61% of the norm and significantly differed from the control ($P < 0.05$) (Fig. 1A–C).

Elevated Plus Maze Test. Diabetic mice demonstrated significant (about twofold) reductions in the number of entries into the open arms and in the time spent in these arms ($P < 0.05$) (Fig. 2A, B). Pretreatment of such mice with propranolol in the respective animal group provided significant increases in the above indices ($P < 0.05$ in comparison with the diabetic group); the number of entries into the open arms corresponded to about 78% of the control, while the recovery in the time spent in these arms was practically complete (Fig. 2A, B). These observations are clearly indicative of a considerable increase in the anxiety level in diabetic mice and nearly full compensation of the respective shifts in propranolol-treated animals.

Light/Dark Board Test. In mice with alloxan-induced diabetes, the numbers of entries and the time spent in the illuminated arena of the light/

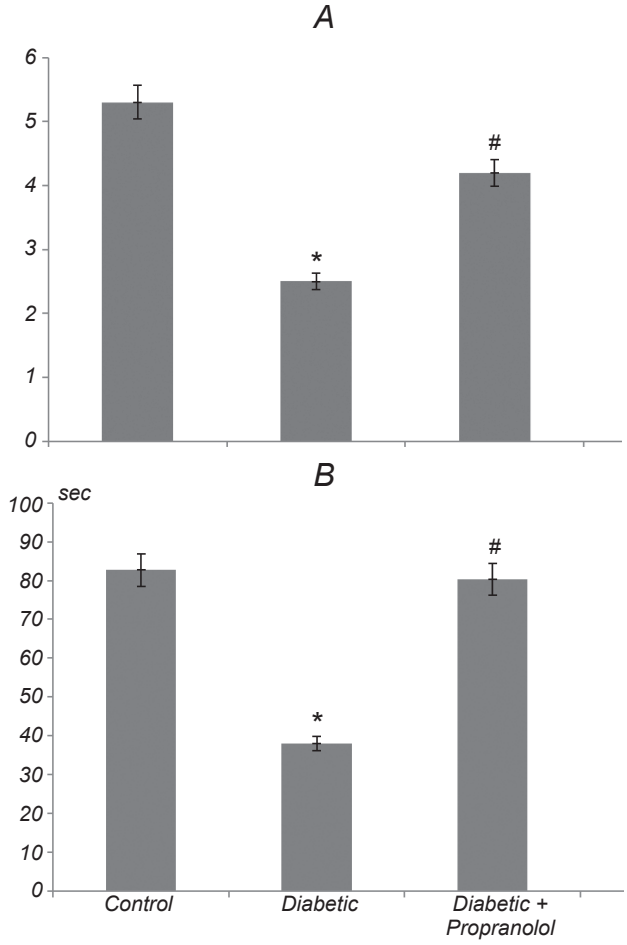


F i g. 1. Effect of propranolol pretreatment on behavioral indices of diabetic mice in the open field test, the intensity of locomotion (A), frequency of rearings (B), and frequency of grooming episodes (C). Experimental groups are indicated below the columns; values are expressed as means ± s.e.m ($n = 6$). Asterisks and # signs show cases of significant intergroup differences with $P < 0.05$ in comparisons of the control vs. diabetic and diabetic vs. diabetic+propranolol animals.

Р и с. 1. Вплив попереднього введення пропранололу на поведінкові показники мишей з діабетом, визначені в тесті відкритого поля (*A* – інтенсивність локомоції, *B* – частота «стійок», *C* – частота епізодів грумінгу).

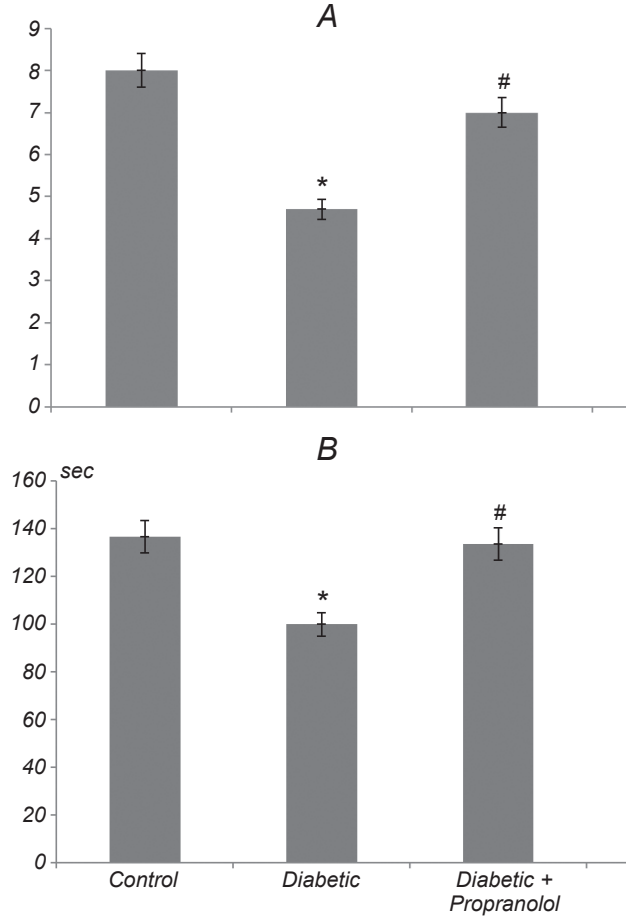
dark box apparatus were significantly smaller as compared with the control (58 and 72%, respectively; $P < 0.05$). Pretreatment with propranolol provided complete or close to complete reversal of these indices ($P < 0.05$, as compared with the group of diabetic mice with no pretreatment) (Fig. 3A, B).

Hole-Board Test. The number of head dips in this test shown by diabetic mice was more than twofold smaller than that in the control ($P < 0.05$). Pretreatment with propranolol provided a significant increase ($P < 0.05$) in the frequency of the head dips in diabetic mice, but the recovery was only partial (to 67% of the norm) (Fig. 4).



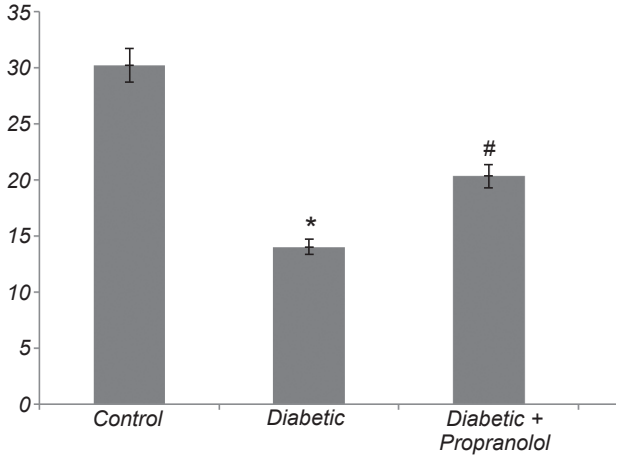
F i g. 2. Effect of propranolol pretreatment on behavioral indices of diabetic mice in the elevated plus maze test, the number of entries into the open arms (A) and time spent in these arms (B). Designations are similar to those in Fig. 1.

Р и с. 2. Вплив попереднього введення пропранололу на поведінкові показники мишей з діабетом, визначені в тесті піднятого лабіринту (*A* – кількість входів до відкритих рукавів, *B* – час, проведений у таких рукавах).



F i g. 3. Effect of propranolol pretreatment on behavioral indices of diabetic mice in the light/dark box test, number of entries into the illuminated arena (A) and time spent in the latter (B). Designations are similar to those in Fig. 1.

Р и с. 3. Вплив попереднього введення пропранололу на поведінкові показники мишей з діабетом, визначені в тесті з освітленим та темним відсіками (*A* – кількість входів до освітленої зони, *B* – час, проведений у такому відсіку).



F i g. 4. Effect of propranolol pretreatment on the behavioral index of diabetic mice in the hole-board test (number of head dips in the holes). Designations are similar to those in Fig. 1.

Р и с. 4. Вплив попереднього введення пропранололу на поведінкові показники мишей з діабетом, визначені в тесті з дошкою з «нірками».

DISCUSSION

The results of our study showed that experimental diabetes in mice causes considerable neurobehavioral alterations. Their majority is, most probably, related to a significantly increased level of anxiety, and this shift can be rather effectively reversed by beta-adrenergic blockade.

Anxiety is a state of cognitive and behavioral preparedness mobilized by an organism in response to a future or a distant potential threat. In its pathological form, excessively high anxiety is a maladaptive state that impairs the ability of an organism to respond optimally. The anxiety level is routinely estimated experimentally using the elevated plus maze, light/dark board, and hole-board tests. Our observations of significant reductions in the number of entries into the open arms of the elevated plus maze, time spent in these arms, and reduction in the number of head dips in the hole-board test are all indices of an elevated anxiety level in diabetic mice. The elevated plus maze and light/dark box test are frequently used for estimation of anxiety-related behaviors in rodents [12]. An anxious animal will systematically prefer dark and confined spaces, as was readily observed in our study.

Shorter times spent in and reduction in the number of entries into the open arm are considered an index of high anxiety [10], thus confirming the anxiogenic role of diabetes under conditions of our study. Significant suppression of the mobility (locomotion), orientational/research activities (rearings), and emotion-related behavior (grooming) shown by diabetic mice in the open field test is, undoubtedly, the consequence of the increased anxiety in such animals. It is, probably, expedient to specially mention the following feature. Behavioral phenomena that are most directly related to orientational/research activity (rearings in the open field test, time spent in the open arms in the elevated plus maze, and inspection of dips in the hole board) were suppressed in diabetic mice most intensely (more than two times as compared with the control).

A general anxiety disorder was reported to be present in 14% of patients with diabetes [13], and, naturally, this disorder needs proper control. Our observations in this study, therefore, provide experimental evidence in support of the use of beta-adrenergic blockers in the case of diabetes-induced neurobehavioral alterations. The expediency of using such type of treatment in clinics needs, however, special further investigations. The clinical relevance of our findings will be a subject of ongoing research in our laboratory.

In conclusion, these results illustrate the protective effect of β-adrenergic blockade in diabetes-induced neurobehavioral alterations in an animal model.

The protocols for animal experiments were conducted in accordance with the guidelines (86/609/EEC) of the European Community Council Directives and those of the Ethics Committee in the University of Ibadan, Nigeria.

The authors of this communication, G. F. Ibironnke and O. S. Asifat, confirm the absence of any conflict related to commercial or financial interests, to interrelations with organizations or persons in any way involved in the research, and to interrelations of the co-authors.

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ВПЛИВИ БЛОКУВАННЯ БЕТА-АДРЕНЕРГІЧНОЇ СИСТЕМИ НА ІНДУКОВАНІ ДІАБЕТОМ ЗМІНИ ПОВЕДІНКИ У МИШЕЙ

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Р е з ю м е

Оцінювали нейроповедінкові показники в трьох групах білих мишей-самців; використовували тест відкритого поля, припіднятий лабіринт, тест-пристрій з освітленим та темним відсіками та дошку з «нірками» (отворами). Миші контрольної групи були інтактними, а в двох інших групах індукували розвиток діабету (одноразові внутрішньоочеревинні ін'єкції 120 мг/кг аллоксану). У третій групі мишам перед уведенням аллоксану внутрішньоочеревинно ін'єкували 40 мг/кг неспецифічного антагоніста бета-адренорецепторів пропранололу. У мишей із діабетом усі тестовані нейроповедінкові показники в чотирьох згаданих тестах були істотно ($P < 0.05$) меншими, ніж такі в контрольній групі. Частота стійок і епізодів грумінгу в тесті відкритого поля, кількість входів до відкритих рукавів піднятого лабіринту та час, проведений у таких рукавах, а також кількість зазирань у «нірки» у відповідному тесті демонстрували найзначніші зниження (більше ніж дворазові). Попереднє введення пропранололу призводило до істотної ($P < 0.05$) нормалізації всіх вказаних нейроповедінкових феноменів; така нормалізація щодо інтенсивності локомоції, частоти грумінгу, часу, проведеного у відкритих рукавах лабіринту, та обох показників у тесті з двома відсіками була практично повною. Отже, діабет у використаній моделі супроводжується розвитком ненормально високої тривожності. Активність бета-адренергічної системи істотно залучена у формування такого стану; фармакологічне блокування бета-адренорецепторів забезпечує істотні анксіолітичні ефекти.

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