DOI 10.1007/s11062-018-9747-x
Neurophysiology, Vol. 50, No. 4, August, 2018

Doxorubicin-Induced Cardiomyopathy in Rats: Behavior of the Animals in the Open Field

O. G. Rodynskii,[1] Yu. V. Kozlova,[1] S. V. Kozlov,[1] G. O. Rodynska,[1] and L. V. Sapozhnychenko[1]

Received June 20, 2017

Under conditions of the open field test, 30 control Wistar rats and 30 rats with experimental cardiomyopathy (CMP) were examined with recording of four behavioral indices, (i) number of crossed squares (intensity of locomotion), (ii) number of rearings (vertical stands), (iii) number of examined holes in the arena, and (iv) number of grooming episodes, within a 28-day-long observation period. The CMP state was induced by five i.p. injections of 5.0 mg/kg of doxorubicin with one-week-long intervals. As was found, all behavioral indices of control rats demonstrated considerable (sometime statistically significant) variations within the observation period (this fact has probably attracted insufficient attention in analogous experiments with long-lasting observation periods). The development of doxorubicin-induced CMP resulted in considerable suppression of all types of behavioral activity, relatively moderate within the first week of observation and dramatic within late phases of the observation period. The dynamics of the numbers of rearings and grooming phenomena were complex, with noticeable increases on days 3 and 14. The dynamics of the numbers of rearings and inspection of the holes in both experimental groups were significantly dissimilar, which indicates that these behavioral phenomena are related to different aspects of the research/orientatinal activity. In general, a state of increased anxiety followed by the development of a depression-like state was observed in CMP rats. These negative shifts in behavioral activity are believed to result from general CMP-induced insufficiency of blood supply of the brain and development of hypoxia in the latter; specific direct effects of doxorubicin on some cerebral structures seem to be unlikely.

Keywords: doxorubicin, experimental cardiomyopathy, open field, behavioral indices

INTRODUCTION

Heart diseases are the main cause of mortality in people of mature age [1]. Cardiovascular pathologies are frequently accompanied by cognitive disorders, neurotic states, psychopathies, and personality disorders [2, 3]. Such disorders, in turn, worsen the prognosis of the underlying disease and, in many cases, lead to the disability of cardiological patients [1].

Disorders in CNS functioning resulting from cardiovascular pathologies have, in general, insufficiently been studied at present. This problem was subjected to intense investigations during many years, but a number of its aspects have still not been sufficiently interpreted; this is why the respective studies remain at present highly urgent [4]. Investigations within this field made necessary the development of adequate animal models of cardiomyopathy (CMP). Among most extensively used models of this pathology, there is doxorubicin-induced CMP. As was found, the antibiotic doxorubicin, in the case of its course introduction, evokes intense side effects manifested as strong pathological changes in the myocardium. The technique of induction of experimental doxorubicin-induced CMP has been developed in detail on rats [5]; the mechanisms of pathogenesis and functional disorders developing under such conditions demonstrate significant similarity to those in humans [6]. As is believed, the main link of the pathogenesis of cognitive disorders against the CMP background are negative changes in blood supply of the brain resulting in the development of circulatory hypoxia [7]. Some researchers, however, supposed that behavioral and cognitive disorders at CMP are related to some direct action of doxorubicin inducing specific structural/functional shifts in the cerebral structures responsible for learning, motor activity,

[1] Dnipropetrovsk State Medical Academy, Ministry of Public Health of Ukraine, Dnipro, Ukraine
Correspondence should be addressed to Yu. V. Kozlova (e-mail: kozlova_yuv@ukr.net)

and spatial orientation [8]. This is why obtaining of new information on behavioral modifications under conditions of CMP is obviously urgent. At present, the situation related to this problem is somewhat paradoxical. The pathophysiological and biochemical changes induced by doxorubicin in the organism of experimental animals have been studied by a number of authors, and the respective information is, from many aspects, relatively detailed. At the same time, behavioral manifestations that accompany experimental CMP were examined only in a few studies. This is why we subjected control rats and those suffering from experimental doxorubicin-induced CMP to testing under open field conditions.

METHODS

The experiments were carried out on 60 albino male Wistar rats (age 5 to 7 months, body mass 180–220 g). We took into account that these animals are most suitable for reproduction of the model of doxorubicin-induced CMP, and the technique of modeling has been developed in most detail precisely on rats [5]. The development of CMP in rats is relatively rapid and demonstrates sufficient similarity to that in humans [6]. Complications of CMP looking as functional disorders in the CNS are based on the same pathogenetic links as those in humans [6].

All animals were divided into two equal groups (n = 30 in each). Rats of the first control group were i.p. injected with 0.9% physiological saline. Animals of the experimental (CMP) group were also i.p. injected with 5.0 mg/kg body mass of Doxorubicin Pharmachemie B. V. (TEVA Pharmaceutical Industries, Netherlands/Israel). Such injections were performed five times with one-week-long intervals.

Examinations of the behavioral indices in the CMP group were performed on days 1, 3, 7, 14, 21, and 28 after the last doxorubicin injection. Rats of the control group were examined synchronously. Selection of the terms of examination was related to periodization of the development of morphological and functional changes in the myocardium; the symptomatology of the respective disorders corresponded to that described earlier [9].

A nearly standard method of observation in the open field was used. The dimensions of the open field arena were 100 cm × 100 cm; the walls were 40 cm high. The set was made from wood and painted with a stable paint. The arena was divided into 25 equal 20 × 20 cm squares. On crossings of the borders of each square, there were openings (diameter 10 mm). Spontaneous individual behavioral activity in the open field was recorded within a 3-min-long observation interval. For each animal, we calculated (i) total number of crossed squares, (ii) number of raisings of the animal on its hindlimbs (vertical stands), (iii) number of inspected holes in the arena, and (iv) that of the grooming episodes [10, 11]. Crossing of the square was taken into account when all four limbs crossed the square borders. Rearings (vertical stands) were taken into account when those were performed both with leaning on the wall and without such support. In the calculations, both short and long-lasting episodes of grooming were taken into account.

Statistical treating of numerical results of observations was performed using STATISTICA v. 6.1 software (StatSoft Inc., U.S.A.; license No. AGAR909E415822FA). Means, s.d., and s.e.m. values were calculated. For intergroup comparisons, the Student's t- and Mann–Whitney U-criteria were used. Both natural values of the indices in both groups and normalized values (%) for the experimental (CMP) group were taken into account (see below).

The relations between the variables and the factors affecting them were estimated by calculation of the coefficient of canonic correlation, R_C, for the dependence between subsets of the data in general within all stages of observation. Results were considered statistically significant at $P < 0.05$.

RESULTS

Before injections of doxorubicin in the CMP group, testing of rats of both CMP and contrl groups demonstrated that there were no significant intergroup differences in the behavioral indices shown in the open field.

The first fact that has attracted our special attention when analyzing the results of tests in the open field was that values of the measured behavioral indices in the control group were found to be noticeably unstable within the 28-day-long observation period. All four analyzed indices demonstrated clear dynamics, and the latter were rather specific for each behavioral manifestation.