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Effects of Phenibut and Citrocard on Non-Competitive and Competitive Behavior during Provoked Aggression in Animals

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Anti-aggressive effects of phenibut (25 mg/kg) and its structural analogue citrocard (50 mg/kg) were revealed in rats under condition of provoked intraspecific aggression. These substances significantly decreased manifestations of aggression in animals: they increased the latency of attacks and reduced their number. Anti-aggressive effects of citrocard were more pronounced than effects of phenibut under conditions of non-competitive aggression induced by fear of inescapable painful exposure or under conditions of competitive aggression reflecting the ability of animals to reveal adaptive social communicative skills in aversive situation.

Key Words: *aggressive behavior; provoked intraspecific aggression; anti-aggressive therapy; phenibut; citrocard*

Aggressive behavior (AB) is a normal species-specific adaptive response typical of various biological species [1,2,4]. From the pharmacological viewpoint, pathological forms of aggression should be corrected, because AB and antagonistic behavior play a role in a variety of mental and nervous diseases and are typical consequences of brain injuries, certain brain tumors, and some other diseases [4,12].

Substances with depressing effects (neuroleptics, tranquilizers, sedatives, and antidepressants) can suppress AB [1,4,9,13]. However, antidepressants are not always effective for AB correction [9]. Neuroleptics, tranquilizers, and sedative substances are mostly used for urgent arrest of aggressive outbursts, but not for long-term treatment, because depressive effects significantly impair quality of life of patients [12]. Phenibut, a GABA analogue, produces anti-aggressive effect without depressing effects [9,10]. Its anticonflict action was a key factor for its inclusion into the ambulance set for astronauts in 1975, because this effect is necessary for correction of social interactions in group of people under conditions of long-term isolation during space flights. Pharmacologists of the Volgograd State Medical University and chemists of A. I. Gertsen Russian Pedagogical University developed a structural analogue of phenibut (citrate of 4-amino-3-phenylbutanoic acid; RGPU-147; citrocard), which nootropic, anxiolytic [8], neuroprotective [7], and cardioprotective [3] activities are more pronounced that activities of phenibut.

Here we compared the effects of phenibut and citrocard on competitive and non-competitive AB of animals during provoked intraspecific aggression.

MATERIALS AND METHODS

Experiments were performed in accordance with the Principles of Good Laboratory Practice (GOST R-53434-2009) and rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and approved by the Regional Independent Ethic Committee (Volgograd State Medical University). Investigations were conducted on outbred male rats aging 3.5-4 months (180-220 g; obtained from Rappolovo Breeding Center of the Russian Academy of Medical Sciences) kept under standard vivarium conditions.

Substances were dissolved in 0.89% NaCl and intraperitoneally injected to animals 45 min before the

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tests. We used phenibut in a dose of 25 mg/kg producing no sedative effect in animals [9]. Citrocard was injected in an experimentally proven effective dose of 50 mg/kg [3]. The substances were synthesized at the Department of Organic Chemistry, A. I. Gertsen Russian Pedagogical University.

Models of anticompetitive and competitive AB were used for estimation of anti-aggressive properties of the test agents.

The model of anticompetitive AB was performed as a provoked interspecific aggression [5]: exposure of animals to electric shock served as provoked aggressogenic stimulus. A pair of animals from one experimental group was placed in a Plexiglas box (27.5×27.5×40 cm) with electrode floor. Alternate current was delivered to the floor for 3 sec (1-sec interval), its voltage was gradually increased (starting from 20 V). Paws were wetted before placing the animal to electrode floor. In case of the absence of aggressive responses after 3 presentations of the stimuli, voltage was increased by 1 V and stimulation was continued until aggression appeared. Voltage inducing aggression after presentation of 3 stimuli of same intensity was considered as a threshold of provoked aggression. Aggressive reaction included rearing with attempts to bite and to hit the partner with fore paws. After 24 h, animals were placed to the box again, and the threshold voltage was put to the floor continuously for 2 min. Latent period (LP) of first attack (sec) and number of attacks were registered. Short (<5 sec) and long-term (5-20 sec) attacks were registered separately for estimation of the intensity of aggressive responses. An increase in aggression threshold and LP of first attack, and reduction of attack number indicated anti-aggressive effects of substances.

Competitive AB was modeled under conditions of provoked intraspecific aggression [5]: the opportunity to escape painful exposure on a safe platform in the center of the box with electrode floor served as the motivation stimulus. Competition for opportunity to escape aversive factor served as aggressogenic stimulus [1,2,4]. On day 1 of the experiment (without administration of the agents), the rats were trained to avoid painful exposure on the safe platform (LP of avoidance was less than 10 sec in 100% cases). On day 2, a pair of rats was placed in the box, threshold current was delivered to the floor for 2 min. LP and duration of joint escape, LP of first attack, and number of attacks were registered. An increase in the LP of first attack and duration of joint escape, and reduction of attack number and LP of joint escape served as criterion of anti-aggressive effects of the agents.

As painful exposure serves as provoking aggressogenic stimulus in all described methods, the indirect effects of the substances on aggression via influence on nociception can not be excluded. Effects of the test substances on nociception of animals were studied in tests of paw stimulation with electric current until vocalization and tail withdrawal from hot water [6].

Statistical analysis of the results was performed using one-way rank Kruskal–Wallis test and Newman–Keuls criterion.

RESULTS

In the model of non-competitive AB during provoked intraspecific aggression in animals, painful exposure promoted agonistic behavior (attacks), a natural protective response to aversive stimulus [4,13]. Threshold current was followed by aggressive impulse attacks in animals: jumping and biting of partner rat, lateral and vertical postures with aggressive attacks and strikes with forelegs. Continuous exposure of animals to aggression-inducing stimulus for 2 min was followed by two types of aggressive attacks: short-term "jump and bite" and "jump and strike" (less than 5 sec) or long-term attacks (5-20 sec) consisting of a series of jumps, bites, and strikes with short intervals (1-3 sec). Separate registration of short-term and long-term attacks allowed differentiating various intensities of attacks. Long-term attacks, especially attacks with destruction, characterize high level of aggression. AB against stocks was also registered: animals bite bars of the electrode floor and attack walls of the cage. In some cases, manifestations of threatening and horrification were found (long threatening vertical postures or passive defeating behavior, *e.g.* escaping, freezing, and spinal postures). Threshold of aggressive response (Fig. 1, *a*) and LP of aggressive reactions (Fig. 1, *b*) were significantly higher in rats receiving citrocard comparing to control group. Effect of phenibut was similar, but less pronounced. In addition, the number of short-term and long-term attacks decreased in animals receiving phenibut and citrocard, and the number of short-term attacks was higher (Fig, 1, *c*). These data reflect the ability of the test substances to suppress aggression in rats.

In the model of competitive AB under conditions of provoked intraspecific aggression during exposure to electric stimuli with threshold voltage, control animals preliminary trained to escape painful exposure on the safe platform started to fight for the platform, though it was big enough for both rats. During competitive fighting for the safe platform, the rats performed short aggressive attacks; long-term attacks were not registered on this model of aggressive interactions. Citrocard and to a lesser extent phenibut exhibited anti-aggressive effects on this model of AB: the number of attacks and LP of joint escape (joint staining of both rats on the safe platform) significantly

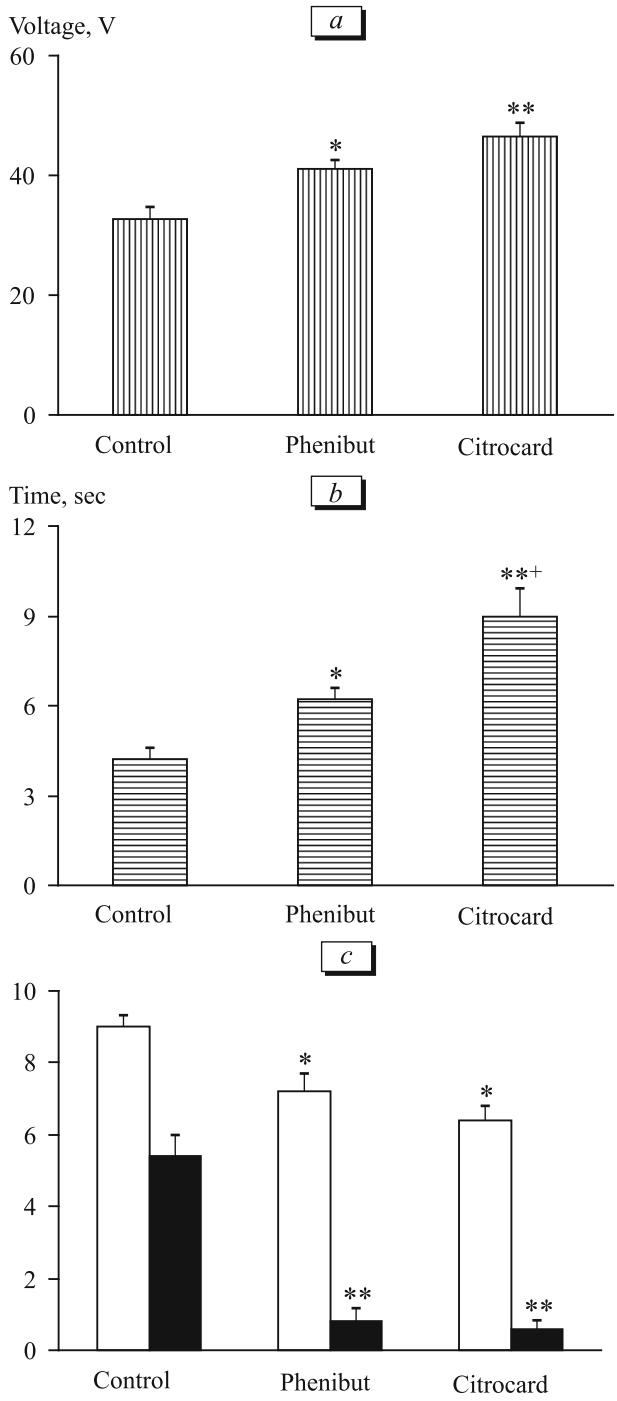

Fig. 1. Effects of phenibut and citrocard on animal behavior in the model of non-competitive provoked interspecific aggression. *a*) Threshold of aggressive reaction; *b*) LP of first attack; *c*) number of short-term (light bars) and long-term (dark bars) aggressive attacks. Here and in Figs. 2, 3: $^{*}p<0.05$, $^{**}p<0.01$ in comparison with the control; $^{+}p<0.05$, $^{++}p<0.01$ in comparison with phenibut.

decreased, and LP of the first attack and duration of joint escape increased after treatment with both agents (Fig. 2). Effects of citrocard on the majority of these parameters were more pronounced than the effects of phenibut.

In the test of foot-shock stress up to vocalization level, citrocard and to a lesser extent phenibut increased the threshold level inducing vocalization in animals in comparison with control rats (Fig. 3, *a*). Hence, both agents suppress pain sensitivity. In the tail-flick test, phenibut and citrocard did not significantly affect LP, *i.e.* did not affect the pain threshold for thermal stimulus (Fig. 3, *b*).

Both models of AB used in the experiment simulate provoked intraspecific impulsive aggression with pain as a provocative agent. However, defensive aggression induced by fear of inescapable pain exposure is typical for the first model, and competitive aggression related to conflict for safe territory, which allows to avoid painful stimulus, is typical for the second model [4].

As in the model of non-competitive AB the rats cannot avoid painful (aggression-inducing) stimulation, anti-aggressive effect of the substances can be related to their effects on nociception system, *e.g.* their analgetic effect. It should be emphasized that citrocard and phenibut suppressed the response to pain, which is mediated by the CNS and especially emotiogenic structures (foot-shock stress up to vocalization level), and produced no analgesic effect in the test measuring pain reaction mediated by peripheral reception and spinal reflexes (tail-flick test). These differences in the effects of citrocard and phenibut during modeling of pain induced by electric and thermal stimuli can be related to the central mechanisms of their action. Indirect effects of the test substances on pain sensitivity of animals via changing the emotional response to pain can not be excluded. It was previously shown that phenibut and citrocard have anxiolytic and antidepressive effects related to the influence to emotiogenic structures of CNS [7,8] and can suppress motivation to AB appearance.

During modeling of competitive aggression, the painful stimulus promotes AB intensification, but reduced sensitivity to pain under these conditions is unlikely associated with the attempts of the animals to avoid painful factor on the safe platform together with another specimen, which serves as a direct object for aggression for self-defense. It can be hypothesized that the anti-aggressive effect of phenibut and citrocard shown in the model of competitive aggression is a result of stimulation of adaptive social behavior and increased ability of the animals to effective communications under aversive conditions. It should be noted that phenibut and citrocard exhibit nootropic activity [7,8], which can promote better training of animals to avoid painful stimulus on the platform.

Structural similarity of citrocard and phenibut and similar neuropsychotropic effects (anxiolytic, antidepressive, nootropic [3,7,8], anti-aggressive, and central analgesic actions) suggest common mechanisms of

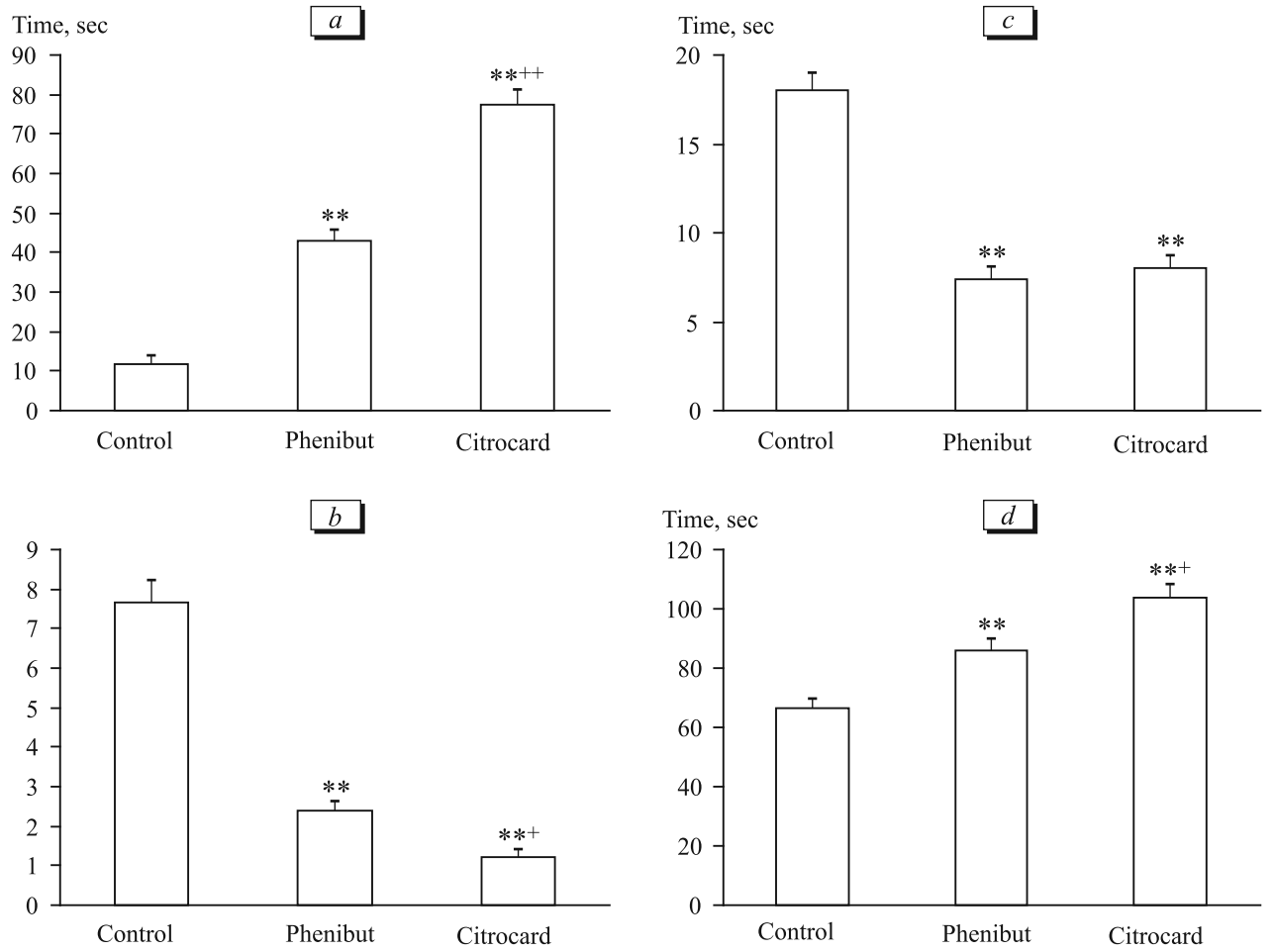

Fig. 2. Effects of phenibut and citrocard on animal behavior in the model of competitive provoked interspecific aggression. *a*) Threshold of aggressive reaction; *b*) number of aggressive attacks; *c*) LP of first joint avoidance; *d*) duration of joint avoidance.

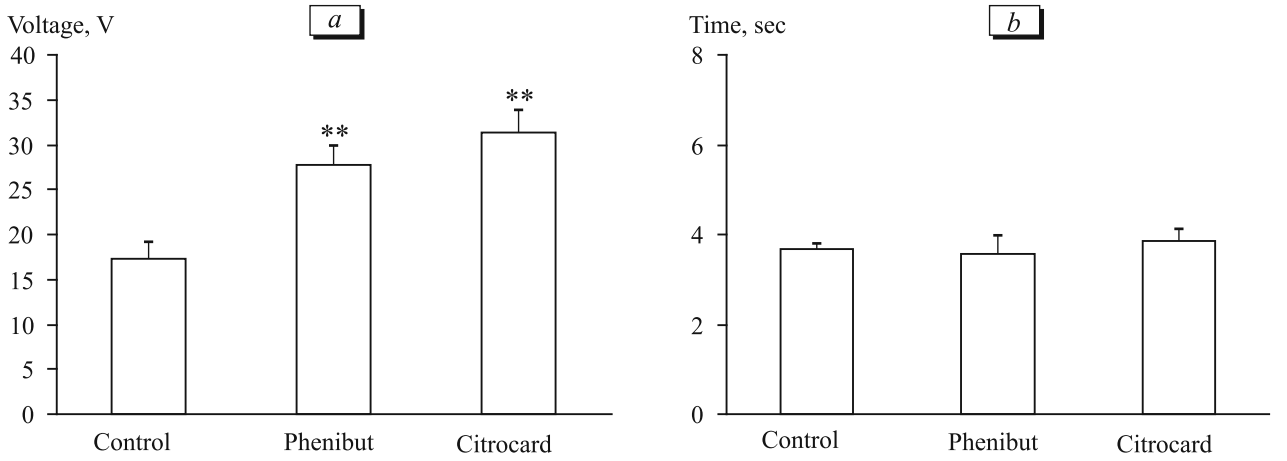

Fig. 3. Effects of phenibut and citrocard on pain sensitivity in animals. *a*) Vocalization threshold; *b*) LP of tail withdrawal.

action of these agents, which can be related to the influence on the CNS. Phenibut is a non-selective agonist of inotropic $GABA_A$- and metabotropic $GABA_B$-receptors [9,10]. The GABA system contributes to neurochemical control of AB: a negative correlation between the activity of GABAergic neurotransmission and AB is shown in animals. It is observed that benzodiazepines and muscimol suppress AB via stimu-

lation of $GABA_A$-receptors [11,12,15]. Contradictory published reports on the contribution of $GABA_B$ receptors into AB regulation are shown. On the one hand, stimulation of $GABA_B$ receptors by microinjections of selective antagonist baclofen in the dorsal raphe nucleus promotes AB activation [14], but microinjections of muscimol induce a reduction of animal aggression [15]. Other authors show that intraperitoneal injections of selective antagonist of $GABA_B$ receptors baclofen and non-selective agonist of $GABA_A$ and $GABA_B$ receptors phenibut to mice suppress aggressive behavior. Under these conditions, baclofen has more specific action, and decrease the frequency and duration of aggressive attacks, and phenibut induces non-specific inhibition of behavioral activity [9,10]. Thus, it can be concluded that anti-aggressive effects of phenibut and baclofen have common mechanisms, which can probably be related to their GABAergic action.

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