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Experiments on the influence of organic compounds with nitrogen and their compositions on the emotional-behavioral reactions of laboratory animals under formalin edema

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ABSTRACT

The experiment on laboratory animals explores their reference and exploratory activity while injecting organic compounds with nitrogen: caffeine paracetamol and carbamazepine and their compositions under formalin edema. The analysis of the results of our experiments shows that caffeine potentiates horizontal and vertical impellent activity and stimulates rats' cognitive activity.

Keywords: paracetamol, caffeine, carbamazepine, "open field", formalin edema, pharmacological composition.

INTRODUCTION

The burning issue at the present period of advance in medicine lies in developing combined medicaments with pharmacological effects, which can be achieved by rational combination of their ingredients. Several components, combined in one medical product, broaden its pharmacological range [1]. Clinical researches have confirmed the advantages of combinations over mono-medicines in pain pharmacotherapy [2]. The advantage of combined medicines over mono-medicines lies in the fact that they are more effective than every separate component in removing pain or inflammation [2]. Combinations like that make it possible to add active materials to medicines in a smaller dose, thus reducing toxicity and negative side effects [3]. Caffeine is known to aggravate analgetic action in nonnarcotic analgetics (NNA) and nonsteroid anti-inflammatory drugs (NAIDs) [4-8]. The probability of a medical composition to produce a more powerful pharmacological action as compared to every separate component lay in the foundation of our experiment.

Pharmacological compositions of NNA and NAIDs of various chemical structure with caffeine have been developed by the Department of Medical and Bioorganic Chemistry at Kharkiv National Medical University (KhNMU) under the guidance of Prof. Syrovaya A. O. The quantum chemical research and an experiment have been conducted to study the influence of organic compounds with nitrogen: caffeine (1,3,7-trimethylxanthine) on the pharmacological activity of NAIDs; paracetamol (para-acetylaminophenol) and carbamazepine (benzo[b][1]benzazepin-11-carboxamid) (a secondary analgesic) on laboratory animals (mature WAG-line rats) by intro-gastric injection.

Nervous, humoral and immune regulations underlie interaction between animals and the environment. Depending on genetically determined peculiarities of these processes, animals react to changes in the environmental conditions in different ways. This fact allows us to single out different individual-typological features in them [9]. Moreover, animals' individual peculiarities are determined by their general state at the moment of finding themselves in extreme conditions, as well as by their previous experience of solving stress situations and other features, acquired during their lifetime.

The purpose of our research was to study the influence of organic compounds on the CNS taking into consideration emotional-behavioral reactions (EBRs) in rats, tested in the "open field" by mono-injection and with pharmacological combinations under formalin edema.

MATERIALS AND METHODS

While studying the mechanism of the effect of pharmacological products it is vital to explore their influence on the behavioral characteristics in animals. That we have no verbal contact with animals limits the range of possible tests. The so-called "open field" test is the most wide-spread and informative one in studying the influence of medicines on the EBRs [10]. The influence of experimental drugs on the rats' EBRs by mono-injection and in the composition with caffeine was studied under formalin edema.

An experimental study was conducted on 54 white WAG-line rats of both sexes with an average weight of 210-230 g. The animals were divided in 9 groups with 6 rats in each group. The animals of group 1 were a control group, receiving 3 % starch mucus once by oral intragastric introduction (2 ml per 200 g rat). Formalin edema in the animals of group 2 was modeled by subplantar injection of 2 % formalin solution in the rat's hind leg along with intragastric injection of 3 % starch mucus [11]. Experimental drugs and their compositions were injected to the animals of 3 through 9 group intragastrically once as a suspension with 3% starch mucilage, namely: paracetamol (30 mg per 1 kg of the animals' weight (mg/kg)) to the animals of group 3, caffeine (0,6 mg/kg) to group 4, carbamazepine (6,25 mg/kg) to group 5, paracetamol (30 mg/kg) combined with caffeine (0,6 mg/kg) to group 6, carbamazepine (6,25 mg/kg) combined with caffeine (0,6 mg/kg) to group 7, paracetamol (30 mg/kg) combined with carbamazepine (6,25 mg/kg) to group 8 and paracetamol (30 mg/kg) combined with carbamazepine (6,25 mg/kg) and caffeine (0,6 mg/kg) to group 9. Maximum development of formalin edema can be observed in a four-hour period after its modeling [11], therefore, medicines, as well as 3 % starch mucus, were injected an hour prior to this moment.

The influence of the medicaments and their combinations on the animals' behavioral characteristics was assessed by comparing groups 3-9 with the control one (group 1), the one under formalin edema (group 2), as well as by mono-injection of experimental products (group 3, 4, 5) under maximum development of formalin edema. Parameters of the rats' reference and exploratory activity were observed during 3 minutes in the "open field" [9] test and by the multi-parameter method of assessing alarming and phobic states according to generally established methods [12]. The parameter of the rats' reference and exploratory activity in the "open field" test is characterized by a series of dimensions: the number of intersected squares (horizontal motion activity (HMA)), upright postures (vertical motion activity (VMA)), observed holes, washings (grooming), urinations and defecations according to generally established methods [10, 13].

The experiments were conducted in accordance with methodical recommendations of the State Pharmacological Center Health Ministry of Ukraine [11]. While choosing the number of animals and classifying them in groups, we took into account economic approach, bioethical rules and statistics requirements. Recalculation of human doses for rats was performed with use of coefficient of species sensitivity according to Rybolovlev Y.R. [14]. Statistical data manipulation was conducted with the help of generally established methods [15]. The experiments were carried out on laboratory animals from the KhNMU experimental biological clinic, taking into account norms of storage, care and feeding, approved in accordance with the principles of the "European Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes" (Strasbourg, 1986) [16] and the resolution of the First National Congress of Bioethics (Kiev, 2007) [17].

The experiments were conducted in the morning, which according to the literature is agreed with the dependence of the main parameters of the test and received pharmacological activity to the study drug by the circadian rhythm [18, 19].

RESULTS AND DISCUSSION

The influence of experimental drugs under mono-injection and their compositions on the rats' HMA and VMA. The analysis of rats' reference and exploratory behavior in the open field test concerning HMA and VMA characteristics showed a motivational component in the rats' characteristic. At the same time they tried to come into indirect contact with objects, located at some distance: the rats sniffed at the objects beyond "the open field".

Table 1 – Indicators of the rats' behavioral activity under formalin edema in the "open field" test (n=6)

№	*Groups of animals*	*Number of intersections (HMA)*	*Number of sets (VMA)*	*Number of peeping into holes*	*Number of washing (grooming)*	*Number of urination*	*Number of defecation*
1.	Control	40,33±1,74	5,67±0,17	1,67±0,21	6,67±1,38	0,67±0,26	4,17±0,40
2.	Formalin edema	57,17±1,25*	7,50±1,77	1,50±0,22	5,17±0,65	1,00±0,00	2,17±0,31 */*****
3.	Paracetamol	38,67±7,73 **/******/*********	5,00±1,73**	1,17±0,17 *	20,17±7,20 */**	1,50±0,22 */**/******/*********	2,00±0,37 *
4.	Caffeine	42,33±7,61 **/******/********/*********	6,17±2,01*****	1,50±0,50	7,83±1,82	1,33±0,33 *********	2,17±0,65 *
5.	Carbamazepine	7,17±2,50 */**/***/****/******/*******/********/*********	2,00±0,80 */**/***/******	1,00±0,00 */**	12,67±8,76	1,00±0,00 ****	1,33±0,33 */**
6.	Paracetamol + caffeine	43,00±8,41 *****/********/*********	8,83±3,46 *****/*********	1,33±0,33	13,83±7,33	1,17±0,17 *********	2,67±0,76
7.	Carbamazepine + caffeine	25,17±8,04 */**/*****	2,33±1,03 */**/****	1,00±0,00 */**	9,00±6,11	1,17±0,17 *********	2,33±0,49 *
8.	Carbamazepine + paracetamol	25,83±2,21 */**/***/*****/******/*********	3,50±0,56 */**	1,00±0,00 */**	12,17±3,94 *********	1,17±0,17 *********	2,33±0,40 *
9.	Carbamazepine + paracetamol + caffeine	17,17±3,36 */**/***/****/******/*******/********/*********	1,83±0,97 */**/***/*****/******	0,00±0,00	3,17±1,69 ***/*****/*********	0,33±0,37 ***/*****/******/*******/********	1,33±0,87*

*Note (average ± error of mean): * – veracity of the results as compared to the control group, P < 0,05; ** – to the rats under formalin edema, P < 0,05; *** – to the rats with mono-injection of caffeine, P < 0,05; **** – to the rats with mono-injection of paracetamol, P < 0,05; ***** – to the rats with mono-injection of carbamazepine, P < 0,05; ****** – to the rats with mono-injection of paracetamol + caffeine, P < 0,05; ******* – to the rats with injection of carbamazepine + caffeine, P < 0,05; ******** – to the rats with injection of carbamazepine + paracetamol, P < 0,05; ********* – to the rats with injection of carbamazepine + paracetamol + caffeine, P < 0,05.*

Modeling of the formalin edema (group 2) contributed to statistically veritable increase in the HMA by 1,4 times and tended to increase the VMA in the rats as compared to the control group (table 1). Mono-injection of paracetamol and caffeine under formalin edema contributed to a statistically veritable increase of the HMA in 1,5 and 1,4 times and the VMA in 1,5 and 1,2 times (correspondingly) as compared to the animals from group 2. The received data did not differ from the control group statistically. Mono-injection of carbamazepine under formalin edema contributes to a statistically veritable decrease in the HMA and VMA both as compared to the animals of group 2, and to the control group.

The number of the surveyed holes. A variation of the rats' reference and exploratory behavior is the number of surveyed holes, an indicator of the hole reflex, which shows the animal's ability to explore the "open field", in particular, to peep into apertures. The number of the surveyed holes characterizes the rats' cognitive activity. Modeling of formalin edema, mono-injection of paracetamol, caffeine and paracetamol in combination with caffeine did not influence the rats' cognitive activity. Mono-injection of carbamazepine and its compositions with paracetamol and caffeine contributed to a statistically veritable decrease in the rats' cognitive activity in 1,5 and 1,7 times as compared to group 1 and 2 correspondingly. Injection of compositions with carbamazepine, paracetamol and caffeine contributed to a decrease in the rats' cognitive activity (see table 1).

Rats' cosmetic behaviour. Grooming (cosmetic behavior) in rats is an important feature of the animals' behavior in the "open field". Traditionally, rats spend the best part of their time on combing out their bodies as compared to their spatial motion. Grooming is closely correlated with motion activity. Therefore, in studying drugs, we find this Grooming closely correlated with physical activity. Therefore, when studying drugs, we find this behavioral characteristic of special interest. Modeling of formalin edema exposed a decrease in washings in 1,3 times as compared to the control (group 1). While injecting experimental products and their compositions (groups 3-8) under formalin edema, we could see increased grooming as compared to group 2 and the control. Injection of paracetamol and its composition with cabamazepine contributed to a statistically veritable decrease in grooming as compared both to group 1 and group 2. Injection of the composition of paracetamol, carbamazepine and caffeine contributed to a statistically veritable decrease in grooming as compared both to group 1-8 (see table 1).

Diuresis, defecation. It should be noted that the number of urination and defecation is significant to indicate the rats' emotional status. The level of the rats' emotional state of is evaluated by the number of these indicators (see table 1). Modeling of the formalin edema (group 2) contributed to an increase in diuresis in 1,5 times as compared to the control group. While mono-injecting paracetamol, we observed a statistically veritable increase in urinations in 1,5 times comparing to group 2. Mono-injection of paracetamol, caffeine, carbamazepine and their combinations did not lead to statistically veritable changes in the rats' urinations as compared to group 2. Injection of the composition of paracetamol, carbamazepine and caffeine (group 9) resulted in lowering urinations by 3 times as compared to group 2 and as compared to the control (group 1) in 2 times (see table 1).

Modeling of the formalin edema (group 2) encouraged a statistically veritable decrease in defecations by 1,9 times as compared to the control group. Injecting paracetamol, caffeine and its combinations with carbamazepine (group 8) under formalin edema did not influence the number of defecations as compared to the animals from group 2. By mono-injecting carbamazepine, we could observe a statistically veritable decrease in the number of defecations by 1,6 times comparing to the animals from group 2. Injection of paracetamol in the combination with caffeine increased the number of rats' defecations in 1,2 times as compared to group 2. Mono-injection of paracetamol and its compositions with caffeine and carbamazepine resulted led to a statistically veritable decrease in the number of defecations in 1,1 and 1,6 times comparing to the animals from group 2. While injecting combinations of paracetamol, carbamazepine and caffeine, we could observe the tendency towards growing in the number of rats' defecations comparing to group 2 (see table 1).

CONCLUSION

1. Analysis of the results of the influence of paracetamol, carbamazepine, caffeine and their compositions on the rats' EBRs under formalin edema points to the fact that it is expedient to prescribe caffeine, paracetamol and their compositions (relatively to the HMA), caffeine and its compositions with carbamazepine (relatively to grooming) and paracetamol (relative to the VMA).

2. The research presents an experimental basis for reasonable development of a new analgesic and anti-inflammatory pharmacological composition, containing organic compounds with nitrogen, such as: paracetamol, caffeine, carbamazepine.

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