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Experiments on new pharmacological compositions under formalin edema

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ABSTRACT

The experiment on rats explores the influence of nonsteroid anti-inflammatory drugs with various chemical structure, caffeine and their compositions on behavioral emotional reactions under formalin edema. The analysis of results of experimental researches shows, that caffeine potentiates the action of diclofenac sodium and ibuprofen concerning the horizontal and vertical motion activity and grooming in rats under formalin edema.

Keywords: caffeine, diclofenac sodium, ibuprofen, "open field", formalin edema, drug composition.

INTRODUCTION

Medical practice often resorts to combined pharmacotherapy. Several components, combined in one medical product, broaden its pharmacological range [1]. When joined in a multi-componential composition, ingredients gain more powerful pharmacological effects. 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. 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 analgesic action in non-narcotic analgesics (NNA) and nonsteroid anti-inflammatory drugs (NAIDs) [4-6]. Potentiation of analgesic action can be produced by improved bioavailability in NNA when combined with caffeine [7], as well as central cholinergic analgesia, inducted by caffeine [8], and structural similarity between molecules of adenosine and caffeine, which accelerates neurochemical action of the latter by blocking specific P_1 purine brain receptors [9].

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. Though a great number of combinations of NAIDs and NNA with caffeine are known to us, however pharmacological practice lacks pharmacological compositions of diclofenac sodium and ibuprofen with caffeine.

Pharmacological compositions of NAIDs of various chemical structure with caffeine have been developed by the Department of Medical and Bioorganic Chemistry at Kharkov National Medical University under the guidance of Prof. Syrovaya A.O. The quantum chemical research and an experiment have been conducted to study the influence of caffeine (1,3,7-trimethylxanthine) on the pharmacological activity of NAIDs with various chemical structure: diclofenac sodium (D-Na) (sodium salt 2-[2,6-dichlorphenyl)-amino]-phenylacetic acid) and ibuprofen (Ib) ((±)-2(4-isobutyl-phenyl)-propionic acid 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 [10]. 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 situation and other features, acquired during their lifetime.

The purpose of our research was to study the influence of NAIDs of various chemical structure with caffeine on the central nervous system with emotional-behavioral reactions (EBR) in rats, tested in the "open field" by mono-injection and in the combination with caffeine.

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 EBR [11]. The influence of experimental drugs on the rats' EBR by mono-injection and in the composition with caffeine was studied under formalin edema.

An experimental study was conducted on 42 rats of both sexes in WAG average weight of 210-230g. The animals were divided in 7 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 sub-plantar injection of 2 % formalin solution in the rat's hind leg along with intragastric injection of 3 % starch mucus [12]. Experimental products and their compositions were injected to the animals of 3 through 7 groups once as a suspension with 3 % starch mucus, namely: caffeine (0,6 mg per 1 kg of animals' weight (mg/kg)) to animals of group 3, D-Na (5 mg/kg) to group 4, the combination of D-Na (5 mg/kg) with caffeine (0,6 mg/kg) to group 5, Ib (6 mg/kg) to group 6 and the combination of Ib (6 mg/kg) with caffeine (0,6 mg/kg) to group 7. Maximum development of formalin edema can be observed in a four-hour period after its modeling [12], therefore medicines, as well as 3 % starch mucus were injected an hour prior to this moment.

The influence of the drugs and their combinations on the animals' behavioral characteristics was assessed by comparing groups 3-7 with the control one (group 1), formalin edema (group 2), as well as by mono-injection of experimental products (group 3, 4, 6) under maximum development of formalin edema. Parameters of the rats' reference and research activity (RRA) were observed during 3 minutes in the "open field" [13] test and by the multi-parameter method of assessing alarming and phobic states according to generally established methods [14]. The parameter of the rats' RRA 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)), surveyed holes, washings (grooming), urinations and defecations according to generally established methods [11, 14].

The experiments were conducted in accordance with methodical recommendations of the State Pharmacological Center Health Ministry of Ukraine [12]. 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. [15]. Statistical data manipulation was conducted with the help of generally established methods [16].

The experiments were carried out on laboratory animals from the Kharkov National Medical University 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) [17] and resolution of the First National Congress of Bioethics (Kiev, 2007) [18]. The experiments were carried out in the first half of the day, which according to the date corresponds to the dependence between the main pharmacological parameters and pharmacological activity of the drugs taken to the examination and circadian rhythms [19, 20].

RESULTS AND DISCUSSION

The influence of experimental medicines under mono-injection and their compositions on the rats' HMA and VMA. The analysis of rats' EBR in "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 "open field".

Modeling of the formalin edema (group 2) contributed to statistically veritable decrease in the HMA by 2 times (up to 41,50 ± 0,50) and in the VMA in 4 times (up to до 4,50 ± 0,72) in the rats relative to the control group (93,00 ± 0,37 and 17,50 ± 0,99 respectively) (table 1).

Table 1 – Indices of the rats' behavioral activity in mono-injection of the drugs under research and their compositions under formalin edema (n=6)

№	Groups of animals	Motion activity		Pilot studies	Emotional activity		
		HMA	VMA				
		Number of intersections	Number of sets	Number of peeping into holes	Number of washing (grooming)	Number of urination	Number of defecation
1.	Control	93,00±0,37	17,50±0,99	7,00±0,77	24,50±0,43	1,67±0,21	6,00±0,73
2.	Formalin edema	41,50±0,50*	4,50±0,72*	0,50±0,32*	3,50±0,56*	0,33±0,37*	0,50±0,32*
3.	Caffeine	52,00±0,86*/**/***	7,50±0,67*/**/*****/****	3,00±0,85*/**	7,50±0,43*/**/*****	3,50±0,34 */**/***/****/*****/********	8,00±0,95 */**/***/****/*****/********
4.	D-Na	66,00±0,63*/**/*****/******/********	8,50±0,76*/**	3,17±0,54*/**	8,33±0,49*/**	0,83±0,57**	1,33±0,87*
5.	D-Na+caffeine	123,00±0,93*/**/***/****/******/*******	13,00±0,73*/**/***/****	3,33±0,67*/**	10,50±0,62*/**/***/*****	1,17±0,49****	3,17±0,31*/**/****
6.	Ib	73,00±0,93*/**/***/*****/*******	9,00±0,68**	3,50±0,85*/**	9,17±0,95*/**	1,00±0,28*	1,50±0,35*/***
7.	Ib +caffeine	157,17±0,75*/**/***/****/*****/********	22,17±0,60*/**/***/****	3,50±0,72*/**	13,00±0,87*/**/***/*****	1,33±0,21**/****	3,50±0,67*/**/***/*****

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

While resorting to mono-injection of caffeine under formalin edema (group 3), we observed statistically veritable increase of the HMA in 1,5 times (52,00 ± 0,86) and the VMA in 2 times (7,50 ± 0,67) relatively to group 2. Thus, the received data did not achieve reference quantities.

Mono-injection of D-Na (group 4) under formalin edema contributed to a statistically veritable increase in the HMA in 1,6 times and the VMA in 2 times as compared to the animals from group 2. The received data, however, did not achieve reference quantities.

While injecting the composition of caffeine with D-Na (group 5), we could observe a statistically veritable increase of the HMA and VMA in 3 times in the rats as compared both to group 2 and groups 3 and 4 and even the control one (group 1). Mono-injection of Ib (group 6) contributed to a statistically veritable increase of the HMA in 2 times (73,00 ± 0,93) and the VMA (9,00 ± 0,68) as compared to the rats of group 2, although the received data failed to reach reference quantities.

While injecting the composition of Ib with caffeine (group 7), we observed a statistically veritable increase of the HMA in 4 times (157,17 ± 0,75) and the VMA in 5 times (22,17 ± 0,60) in the rats as compared to group 2 and groups 3, 5 and 7 and even the control group (group 1) (see table 1).

The number of the surveyed holes. A variation of the rats' reference and exploratory behavior is the number of surveyed hole, 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 the formalin edema contributed to a statistically veritable decrease in the animals' cognitive activity in 14 times (up to 0,50 ± 0,32) as compared with the control group (see table 1).

Mono-injection of caffeine, D-Na and Ib and their compositions (groups 3-7) showed a statistically veritable increase in the rats' cognitive activity in 6-7 times: 3,00 ± 0,85 (group 3), 3,17 ± 0,54 (group 4) and 3,33 ± 0,67 (group 5), 3,50 ± 0,85 (group 6), 3,50 ± 0,72 (group 7) as compared to group 2. The received data though tended to grow, but failed to achieve reference quantities.

Rats' cosmetic behavior. 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 motion activity. Therefore, when studying drugs, we find this behavioral characteristic of special interest.

Modeling of the formalin edema exposed a statistically veritable decrease in washings up to 7 times: to 3,50 ± 0,56 as compared to the control (24,50 ± 0,43). While injecting caffeine and their compositions under formalin edema, we could observe a statistically veritable increase in grooming as compared to group 2 in 2-3 times (groups 3-5) (see table 1). While injecting caffeine, D-Na, Ib and their compositions under formalin edema, we observed a statistically veritable increase in grooming in 2-3 times: to 7,50 ± 0,43 (group 3), 8,33 ± 0,49 (group 4), 10,50 ± 0,62 (group 5) 9,17 ± 0,95 (group 6), 13,00 ± 0,87 (group 7) as compared to group 2 (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 formalin edema (group 2) contributed to a statistically veritable decrease of diuresis in 5 times (to 0,33 ± 0,37) and defecations in 12 times (to 0,50 ± 0,32) as compared to the control group (to 0,50 ± 0,32). Injection of caffeine under formalin edema contributed to a statistically veritable increase in the indicators under research as compared to the animals from group 2: the number of urinations grew in 2,1 times (to 3,50 ± 0,34) and defecations – in 16 times (to 8,00 ± 0,95). This last result differs from all the experimental groups from the point of statistical significance. Mono-injection of D-Na and Ib did not show statistically significant changes in the rats (urinations and defecations) comparing to group 2. Injection of the composition of diclofenac sodium with caffeine contributed to normalized diuresis, which did not differ from the control group from the point of statistical significance, and contributed to increased defecations as compared to group 2, 4, 6 decreased defecations as compared to group 1, 3 and 7. Injection of the composition of Ib with caffeine contributed to normalized diuresis (1,33 ± 0,21), which did not differ from the control group from the point of statistical significance, and contributed to increased defecations (3,50 ± 0,67) comparing to group 2, 4-6 and decreased defecations as compared to group 1 and 3.

CONCLUSION

1. There has been studied the influence of NAIDs of diclofenac sodium as the derivative of acetic acid, ibuprofen as the derivative of propionic acid, caffeine and their compositions on the rats' EBR under formalin edema.
2. Caffeine potentiates the effect of diclofenac sodium and ibuprofen as compared to the HMA, VMA and grooming in the rats under formalin edema.
3. Injection of diclofenac sodium, ibuprofen, caffeine and their compositions improves rats' cognitive activity under formalin edema. However, addition of caffeine to diclofenac sodium and ibuprofen does not contribute to improving rats' cognitive activity from the point of statistical significance in experimental conditions.
4. Injection of compositions of diclofenac sodium and ibuprofen with caffeine normalizes diuresis and tends to normalize defecation in rats under formalin edema.

The pharmacological combination of ibuprofen with caffeine is the most effective one according to all indicators of rats' reference and exploratory activity. The results of our experiment can serve as a basis for development of new domestic combined medicines.

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