EXPERIMENTAL RESEARCH ON POSSIBLE INTERACTIONS OF HIGH DOSES OF METAMIZOLE SODIUM WITH THE ENDOGENOUS CANNABINOID OR DOPAMINERGIC SYSTEMS

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Abstract

For metamizole sodium the four specific effects of cannabinoids in mice were previously noticed by us (the tetrad): analgesia, sedation, hypothermia, catalepsy.

For verifying the cannabinoid hypothesis, the effects of the association between metamizole sodium 1g/kg bw and a CB1 receptors antagonist (AM281) in the hot-plate test and on body temperature were tested in mice. The results showed that metamizole in high doses possessed a central analgesic and hypothermic effect, but they were not influenced by the CB1 receptors antagonist. These findings suggested that the demonstrated analgesic and hypothermic effects of metamizole do not involve a cannabinoid mechanism.

Another hypothesis regarding the dopaminergic action of metamizole was analysed. Catalepsy was tested in mice treated with metamizole or chlorpromazine alone or associated with bromocriptine. The results showed that the cataleptic effects of metamizole are not due to the blockage of the dopaminergic receptors. Regarding the cannabinoid-like effects of metamizole (the tetrad), further research of other mechanisms of action of metamizole is required.

A hypothesis for the hypothermic effect of metamizole could be its implication in stimulation of the thermolysis process. This effect for metamizole was not demonstrated until now and it may extend its indications area in inducing controlled hypothermia in humans.

Rezumat

În cercetări anterioare, s-au observat pentru metamizolul sodic 4 efecte specifice canabinoizilor la şoareci (tetrada): analgezie, sedare, hipotermie, catalepsie.

Pentru a verifica ipoteza canabinoidă, s-au testat la şoareci efectele asocierii dintre metamizol 1g/kgcorp şi un antagonist al receptorilor CB1 (AM281) în testul plăcii încălzite şi asupra temperaturii corporale. Rezultatele au arătat că doze mari de metamizol au un efect analgezic central şi hipotermizant la şoareci, care nu au fost însă influenţate de antagonistul CB1. Aceste rezultate sugerează că efectele analgezice şi hipotermizante demonstrate de noi nu implică un mecanism canabinoid.

A fost analizată şi ipoteza acţiunii dopaminergice a metamizolului. S-a testat efectul cataleptic la şoareci trataţi cu metamizol sau clorpromazină singure sau asociate cu bromocriptină. Rezultatele au arătat că efectele cataleptice ale metamizolului nu sunt datorate blocării receptorilor dopaminergici. Sunt necesare noi cercetări privind

mecanismele de acţiune ale metamizolului în ceea ce privesc efectele sale canabinoide (tetrada).

O ipoteză privind efectul hipotermizant al metamizolului ar fi implicarea sa în procesele de termoliză. Acest efect nu a fost încă demonstrat pentru metamizol şi i-ar putea lărgi aria indicaţiilor în inducerea hipotermiei controlate la oameni.

Keywords: Metamizole sodium, cannabinoid system, tetrad, dopaminergic system

Introduction

Metamizole sodium is a drug with well-known analgesic, antipyretic and also moderately antispastic and anti-inflammatory effects. Its classic mechanism of action involves inhibition of cyclooxygenases (COX1, COX2). At high concentrations inhibition of COX3 isoenzyme was described [2,9]. There are also studies which show the interference of metamizole sodium with the endogenous opioid system [12].

These different actions of metamizole sodium may be due only to its metabolites because it is detectable in the serum for only about 15 min following intravenous administration [10]. After oral administration and absorbtion it is rapidly hydrolized to 4-methyl-amino-antipirine (4-MAA) and then metabolized in different other metabolites. After oral administration of metamizole, its active metabolite (4-MAA) reaches the peak concentration in 1.2-2 hours and is further metabolized in 3 principal metabolites: 4-AA (4 -amino-antipirine), 4-FAA (4-formyl-amino-antipirine) and 4-AAA (4-acethyl-amino-antipirine) [1]. The analgezic effect of metamizole correlates with the presence of its metabolites and not with the duration of the presence in plasma of the parental drug. The onset and duration of the analgesic effect correlate with saliva concentrations of 4-MAA and 4-AA. These metabolites were considered to be involved in the interferences with the endogenous cannabinoid system. 4-MAA and 4-AA are bound to arachidonic acid in the brain and form arachidonoyl amides of 4-MAA and 4-AA. The arachidonoyl amides of 4-MAA and 4-AA bind to both human recombinant cannabinoid CB1 and CB2 receptors at micromolar concentrations [10].

Membrane cannabinoid receptors are of at least 2 types: CB1 and CB2. Their names are derived from the plant *Cannabis sativa*, whose principal psychoactive component, delta 9 tetrahydrocannabinol (delta 9 THC), actions upon this type of receptors. There are at least 2 endogenous substances which take action upon these ones: anandamide and 2-arachidonoyl glycerol.

Delta 9 THC and all the other cannabinoids and endogenous cannabinoids as well, which action on CB1 receptors, have in mice four

effects well-known under the name of tetrad: central analgesia, hypothermia, sedation and catalepsy. For a long time it was believed that these effects on a whole are specific for substances which action on CB1 receptors. However, some neuroleptics were also described as producing the tetrad (including chlorpromazine) and some dual blocking substances of cyclooxygenase and lipoxygenase, such as phenindione, and also arachidonic acid [13,14]. In 2011, beforehand Rogosch T et al. discoveries [10], we showed that metamizole sodium, in doses of 500-1000 mg/kg body weight (bw) had all four effects characteristic for tetrad in mice [7]. This could be an argument for the mechanism of action of metamizole sodium. The question is if metamizole sodium or its metabolites mentioned above had the four effects of tetrad caused by the interference with the cannabinoid receptors. We chose to test in the presence of a CB1 cannabinoid antagonist two of the effects from the tetrad of the metamizole sodium (the central analgesic effect in the hot plate test and the hypothermic effect, as well). Since the results did not indicate a possible cannabinoid mechanism of action for metamizole sodium, we tried to establish another valid mechanism for the cataleptic effect registered in previous studies. The stimulation of dopamine receptors with bromocriptine was chosen in order to counteract their blockage through neuroleptics, namely chlorpromazine, because this dopaminergic blocking effect was demonstrated to be active in catalepsy induction. The dopaminergic stimulation effect upon the cataleptic action of the metamizole sodium was also evaluated.

Materials and Methods

Three experimental tests were performed, as follows:

Test 1 – the hot-plate test which evaluated the central type analgesic effect of metamizole sodium, administered alone or together with a CB1 cannabinoid receptors antagonist (AM 281);

Test 2 - evaluation of the body temperature, which assesses the hypothermic effect of metamizole sodium administered alone or together with AM281;

Test 3 –evaluation of catalepsy which assesses the cataleptic effect of metamizole sodium and of chlorpromazine administered separately or associated with bromocriptine, a dopaminergic agonist.

Animals

Swiss albino (NMRI) male mice (8-10 animals/group with 20-25g bw) were used. Animals were brought in the laboratory with at least 48 hours prior to the experiments, were maintained in standard housing conditions, with access to food and water *ad libitum* and were kept in

temperature-controlled rooms at 24ºC. All experiments were carried out between 9 AM and 5 PM. They were in accordance with the International Guidelines for Animal Experimentation and had the approval of the Institutional Ethics Committee. Animals came from the biobase of the University of Medicine and Pharmacy Carol Davila, Bucharest.

Substances

Saline, metamizole sodium, chlorpromazine and bromocriptine originated from industrial products as follows: for metamizol sodium the product used was Algocalmin® (Zentiva S.A.), for chlorpromazine (hydrochloride) Plegomazin® (Egis), for bromocriptine (methanesulphonate) Brocriptin® (Biofarm SA). Substance AM 281 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide), methylcellulose powder were bought from Sigma Aldrich and dimethylsulfoxide (DMSO) from Fluka Ind. In our tests these substances were administered as follows: by gavage - bromocriptine, suspended in methylcelulose 2%; by intraperitoneal injection - metamizole sodium and chlorpromazine dissolved in saline; AM 281 dissolved in DMSO: saline 1:3 vol/vol.

Apparatus and measured parameters

1. In the hot plate test, a device with a thermostat was used (Plactronic Selecta), which heats a metal plate with a side of 24 cm at a constant temperature. The temperature used (55 ± 0.5°C) was in accordance with the literature [15].

Limitation of the animal escape from the plate was prevented by using a cilinder with a diameter of 19 cm and a height of 18 cm, height at which the mice could jump. Two parameters were determined: the first reaction (liking of the paws) and the second reaction (jump). Cut off time for jump was 3 minutes (180 seconds). No pre-testing of mice sensibility was performed because of the risk of false results (usually mice remember the noxious stimuli and react exgerately) [4].Testing was made at 2 hours after administration of metamizol sodium and at 30 minutes after administration of AM281, respectively.

2. In the test of body temperature evaluation, this parameter was measured using a RET3 probe (thermocouple) from ADInstruments GmbH, with the following specifications:

- temperature domain from -273 to +125°C;
- time constant of 0,05 seconds;
- sensor – thermocouple type T from copper-constantan;
- diamether of the head sphera of 0.2 cm;

- length of the thermocouple shaft (the handle) of 1.9 cm.

The probe was introduced entirely in the mouse rectum (approximate 2 cm). The measurements were made with the aid of a digital thermometer Acorn® Temp TC Thermocouple model WD-35627-00. Measurements of the rectal temperature were made at 30 minutes and then at 2 hours after administering metamizole sodium and AM281.

3. In the test of catalepsy evaluation, experiments were performed in a Plexiglas box with transparent 40/30/20 cm (length/width/height) box without cover. On the floor of the box a hard deformable cardboard box (4.5/3.5/3cm - length/width/heigth) was mounted. The illumination during the experiments was natural and the noise level in the experimental room was very low. The mice were easily put with the fore legs on the edge of the box so they were placed with their back to the examiner. Their manipulation was performed only by tail in order to avoid as much as possible the so-called „pinch induced catalepsy". The time during which the mouse remained still in that uncomfortable position was measured (with the exception of the respiratory movements). The „cut off time" was of 60 seconds. Measurements were made at 30 and 60 minutes after the administration of the substances which produced catalepsy (chlorpromazine, metamizole sodium). In some groups, pre-treatment with bromocriptine was used in order to reverse catalepsy. Pre-treatment supposed two orally administration of bromocriptine, 24 hours before and 2 hours before administration of chlorpromazine and metamizole sodium. Interpretation of the results was made on the mean of the values registered at the two measurements.

Statistical analysis

The statistical analysis of the results implied the calculation of the mean, standard deviation and standard error. The groups were analysed regarding the homogeneity of the variances (Levene test). Depending on the results (if $p>0.05$ the groups could be considered homogenous), further processing of the results were made using ANOVA and Tukey test (for homogenous groups) or using Tamhane test (for non- homogenous groups). For Tukey and Tamhane tests singificant values were considered to be $p<0.05$. For processing results, Microsoft Excel and SPSS 15.0 were used.

Results and Discussion

The results are shown in tables and figures.

For test 1 the results are presented in table I and in figure 1 (time until the first reaction – liking of the paw) and in table II and figure 2 (time until the second reaction – jump) .

Table I

Results for the first reaction (liking of the paw) in the hot-plate test for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM281 1mg/kg bw) and group IV (metamizole sodium +AM281 1mg/kg bw).

	Group	Mean	Standard error	P value (Tamhane test) depending of the group *			
				I	II	III	IV
Time (seconds)	**Group I (Control Saline + DMSO:SF 1:3 vol/vol)**	**10.65**	0.63		**0.028**	**0.036**	**0.009**
	Group II (Metamizole sodium 1000 mg/kg bw)	**34.46**	6.4	**0.028**		**0.016**	0.932
	Group III (AM 281 1 mg/kg bw)	**8.3**	0.37	**0.036**	**0.016**		**0.005**
	Group IV (Metamizole sodium 1000 mg/kg+AM 281 1 mg/kg bw)	**43.53**	7.27	**0.009**	0.932	**0.005**	

* Non-homogenous variances (Levene test $p<0.05$). ANOVA was not used. Tamhane test can be used.

Table II

Results for the second reaction (jump) in the hot-plate test for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM 281 1mg/kg bw) and group IV (metamizole sodium +AM 281 1mg/kg bw).

	Group	Mean	Standard error
Time (seconds)	**Group I (Control Saline + DMSO:SF 1:3 vol/vol)**	**91.1**	8.11
	Group II (Metamizole sodium 1000 mg/kg bw)	**86.7**	5.51
	Group III (AM 281 1 mg/kg bw)	**88.7**	5.85
	Group IV (Metamizole sodium 1000 mg/kg+AM281 1 mg/kg bw)	**90.5**	8.03

* Homogenous variances (Levene test $p>0.05$). ANOVA can be used. Without statistical significance. Multiple post-hoc comparison tests were not used.

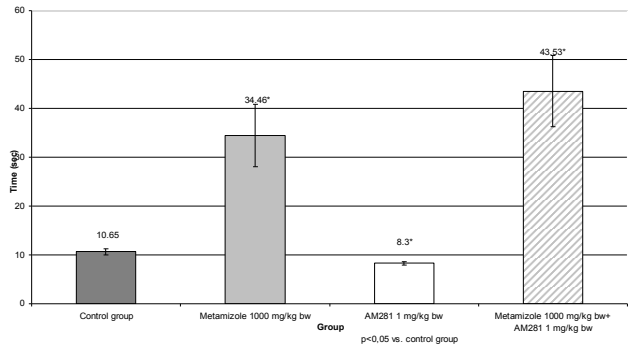

Figure 1

Results for the first reaction (liking of the paw) in the hot-plate test for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM 281 1mg/kg bw) and group IV (metamizole sodium +AM 281 1mg/kg bw). Each column represents the mean value of the time in seconds until the first reaction – liking of the paw (n=10 animals/group). The variance bars represent the standard error. Control group: 10.65±0.63 sec. Group II: 34.46±6.4 sec (p=0.028 compared with control). Group III: 8,3±0,37sec (p=0,036 compared with control, p=0.016 compared with group II). Group IV: 43.53±7.27 sec (p=0.009 compared with control, p=0.005 compared with group III).

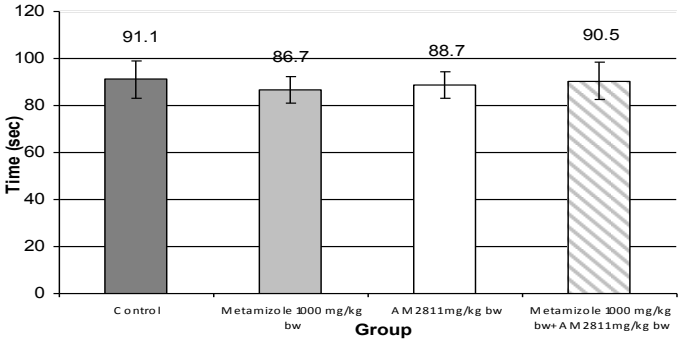

Figure 2

Results for the second reaction (jump) in the hot-plate test for group I (control – vehicle), group II (metamizole sodium 1000 mg/kgbw), group III (AM 281 1mg/kg bw) and group IV (metamizole sodium +AM 281 1mg/kgbw). Each column represents the average value of the time in seconds until the second reaction – jump (n=10 animals/group). The variance bars represent the standard error. Control: 91.1±8.11 sec. Group II: 86.7±5.51 sec. Group III: 88.7±5.85 sec. Group IV: 90.5±8.03 sec. Differences between groups have no statistical signification.

For test 2 the results are presented in tables III and IV. Differences between the temperature at baseline and at 30 minutes after injection of the substances are shown in figure 3. Differences between the temperature at baseline and at 120 minutes after injection of the substances are shown in figure 4.

Table III

Results for the body temperature values measured with an intrarectal thermocouple in mice for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM 281 2mg/kg bw) and group IV (metamizole sodium +AM 281 2mg/kg bw). Temperature values at baseline, at 30 minutes after substances administration and their differences are shown.

Tempera-ture (ºC)	Group	Mean	Standard error	P value (Tukey test) depending of the group *			
				I	II	III	IV
At baseline	**Group I**	**37.83**	0.09				
	Group II	**37.76**	0.07				
	Group III	**37.75**	0.12				
	Group IV	**37.94**	0.1				
At 30 minutes after substan-ces admi-nistra-tion	**Group I**	**35.76**	0.25				
	Group II	**33.66**	0.26				
	Group III	**35.88**	0.23				
	Group IV	**33.18**	0.33				
Differ-rence	**Group I**	**-2.06**	0.29		**<0.001**	0.967	**<0.001**
	Group II	**-4.1**	0.27	**<0.001**		**<0.001**	0.376
	Group III	**-1.88**	0.17	0.967	**<0.001**		**<0.001**
	Group IV	**-4.76**	0.38	**<0.001**	0.376	**<0.001**	

* Homogenous variances (test Levene p>0.05). ANOVA p<0.05. Tukey test can be used.

Table IV

Results for the body temperature values measured with an intrarectal thermocouple in mice for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM 281 2mg/kg bw) and group IV (metamizole sodium +AM 281 2mg/kg bw). Temperature values at baseline, at 120 minutes after substances administration and their differences are shown.

Tempera-ture (ºC)	Group	Mean	Standard error	P value (Tamhane test) depending of the group *			
				I	II	III	IV
At baseline	**Group I**	**37.83**	0.09				
	Group II	**37.76**	0.07				
	Group III	**37.75**	0.12				
	Group IV	**37.94**	0.1				
At 120 minutes after substan-ces admi-nistra-tion	**Group I**	**36.93**	0.22				
	Group II	**32.76**	0.29				
	Group III	**36.98**	0.22				
	Group IV	**32.68**	0.6				
Differ-rence	**Group I**	**-0.9**	0.25		**<0.001**	0.999	**0.001**
	Group II	**-5**	0.35	**<0.001**		**<0.001**	1.000
	Group III	**-0.78**	0.15	0.999	**<0.001**		**0.001**
	Group IV	**-5.26**	0.63	**0.001**	1.000	**0.001**	

*Non-homogenous variances (test Levene p<0.05). ANOVA was not used. Tamhane test can be used.

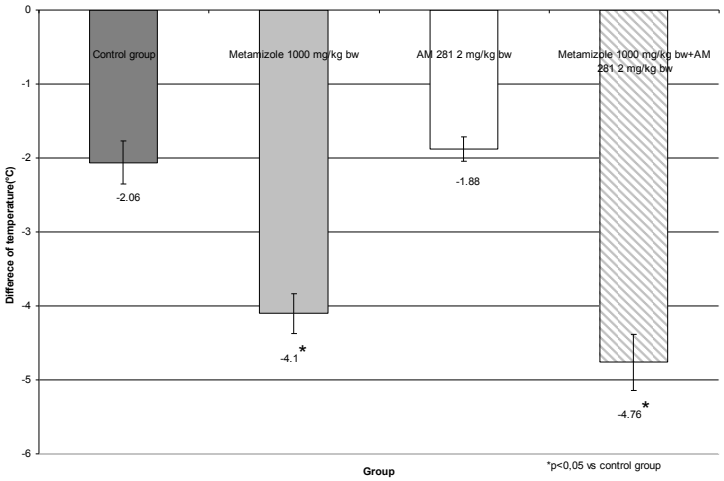

Figure 3

Results for the body temperature values measured with an intrarectal thermocouple in mice for group I (control – vehicle), group II (metamizole sodium 1000 mg/kgbw), group III (AM 281 2mg/kgbw) and group IV (metamizole sodium +AM 281 2mg/kg bw) at 30 minutes after administration of the substances. Each column represents the mean value of the differences between the temperature values at baseline and at 30 minutes after substances administration for each group n=8 animals/group). The variance bars represent the standard error. Control group:- 2.06 ± 0.29 °C; Group II: -4.1 ± 0.27 °C ($p<0.001$ compared with control). Group III: -1.88 ± 0.17 °C ($p<0.001$ compared with group II); Group IV: -4.76± 0.38 ($p< 0.001$ compared with control, $p< 0.001$ compared with group III).

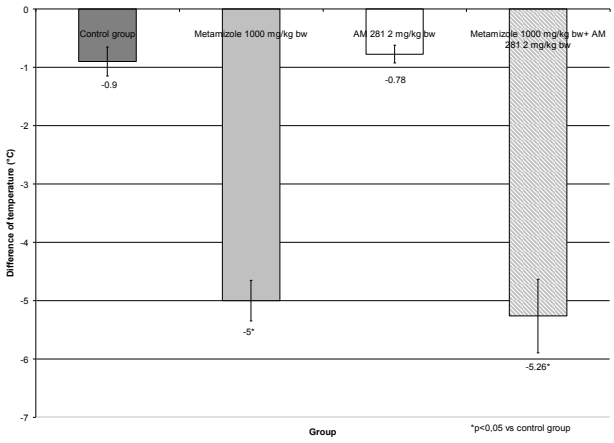

Figure 4

Results for the body temperature values measured with an intrarectal thermocouple in mice for group I (control – vehicle), group II (metamizole sodium 1000 mg/kg bw), group III (AM 281 2 mg/kg bw) and group IV (metamizole sodium +AM281 2 mg/kg bw) at 120 minutes after administration of the substances. Each column represents the average value of the differences between the temperature values at baseline and at 120 minutes after substances administration for each group (n=8 animals/group). The variance bars represent the standard error. Control group: -0.9 ± 0.25 °C; Group II: -5±0.35°C; Group III: -0.78 ± 0.15 °C ($p<0.001$ compared with control); Group IV: – 5.26± 0.63 ($p= 0.001$ compared with control, $p=0.001$ compared with group III).

The results in test 3 are shown in table V. The time during which the mouse remained still in an uncomfortable position was measured (mean value of 2 measurements made at 30 minutes and 60 minutes starting with 30 minutes after substances administration) is shown in figure 5.

Table V

The time in seconds during which the mice remained still in an uncomfortable position for group I (bromocriptine 4 mg/kg bw, 2 administrations), group II (metamizole sodium 1000 mg/kg bw), group III (metamizole sodium 1000 mg/kg bw + bromocriptine 4 mg/kg bw, 2 administrations), group IV (chlorpromazine 5 mg/kg bw) and group IV (chlorpromazine 5mg/kg bw + bromocriptine 4 mg/kg bw, 2 administrations).

	Group	Mean	Standard error	P value (Tukey test) depending on the group *				
				I	II	III	IV	V
Time for maintaining an uncomfortable position (seconds)	**Group I Bromocriptine 4 mg/kg bw, 2 administrations**	**0.5**	0.5		**<0.001**	**0.012**	**<0.001**	0.058
	Group II Metamizole sodium 1000 mg/kg bw	**46.42**	7.57	**<0.001**		0.495	0.942	0.174
	Group III Metamizole sodium 1000 mg/kg bw +Bromocriptine 4 mg/kg bw, 2 administrations	**32**	6.43	**0.012**	0.495		0.155	0.958
	Group IV Chlorpromazine 5 mg/kg bw	**53.08**	4.98	**<0.001**	0.942	0.155		**0.038**
	Group V Chlorpromazine 5 mg/kg bw +Bromocriptine 4 mg/kg bw, 2 administrations	**25.92**	8.52	0.058	0.174	0.958	**0.038**	

* Homogenous variances (test Levene $p>0.05$). ANOVA $p<0.05$. Tukey test can be used.

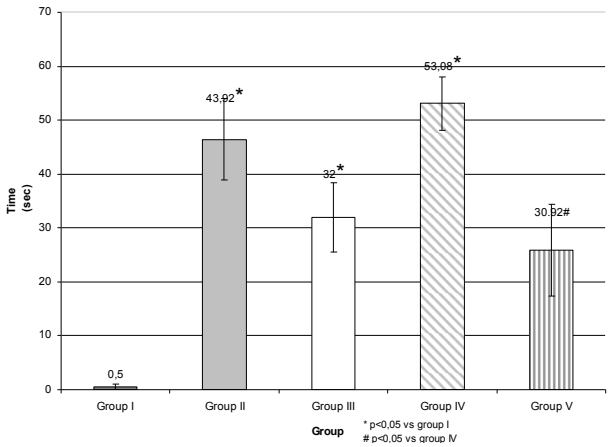

Figure 5

The time during which the mouse remained still in an uncomfortable position was measured (mean values of 2 measurements made at 30 minutes and 60 minutes starting with 30 minutes after substances administration). Cut off time 60 seconds (n=8 animals/group). Five groups of mice were used: group I (bromocriptine 4 mg/kg bw, 2 administrations), group II (metamizole sodium 1000mg/kg bw), group III (metamizole sodium 1000 mg/kg bw + bromocriptine 4 mg/kg bw, 2 administrations), group IV (chlorpromazine 5mg/kg bw) and group V (chlorpromazine 5mg/kg bw + bromocriptine 4 mg/kg bw, 2 administrations). Each column represents the average value of the time the mouse remained still in an uncomfortable positions. The variance bars represent the standard error. Group I: 0.5±0.5 sec. Group II: 46.42 ±7.57 sec ($p<0.001$ compared with group I). Group III: 32±6.43 sec ($p=0.012$ compared with group I). Group IV: 53.08±4.98 sec ($p<0.001$ compared with group I). Group V: 25.92±8.52 sec ($p=0.038$ compared with group IV).

Metamizole sodium, in high doses, 1000 mg/kg bw, presented an analgesic effect in hot plate test, a hypothermic, a cataleptic and a sedative effect (the last tested at a dose of 500 mg/kg bw) These results were reported at the 12th International Congress of the Romanian Society of Clinical Pharmacology, Therapeutics and Toxicology, Bucharest, 2011 [17]. Therefore metamizole sodium meets all the 4 conditions in order to fit the experimental cannabinoid type profile („the tetrad") in mice. The question is if the endogenous cannabinoid system (through stimulation of CB1 receptors) is involved in these effects.

There are few literature data [5,6], regarding the analgesic effect of metamizole sodium in hot plate test.

Preliminary tests made in our laboratory pointed out an analgesic effect for metamizole sodium in hot plate test for the first parameter (liking of the paw). This effect was present only at high doses (1000 mg/kg bw) but

not in lower doses (250 mg/kg bw or 500 mg/kg bw). These results might be predictable because the hot plate test has a high specificity but a small sensibility for analgesia. Generally, substances from the group of non-steroidal anti-inflammatory drugs (NSAIDs) modify especially the latency for paw liking but not the jump time.

The dose of 1000 mg/kg bw of metamizole sodium is a very high dose, relative to the LD50 found in Toxnet (250 mg/kg bw intraperitoneally and 2891 mg/kg bw orally, both in mice). In our experiments, for the mouse strain used, no deaths were registered during testing.

In test 1 (hot plate test), we obtained an analgesic effect in group II (treated with metamizole sodium 1000mg/kg bw) and also in group IV (treated with metamizole sodium 1000mg/kg bw associated with AM281 1mg/kg bw), without any difference between the two groups. In group III (AM281 1mg/kg bw) an algogenic effect for the first reaction of the animal (liking of the paw) was registered. We suppose that a cannabinoid tonus was focused and it functioned by reducing the perception of pain, the reduction of this tonus determining an algogenic response, also reported in previous papers [8]. Nevertheless, the analgesic effect of metamizole sodium was not significantly reduced by the association of AM 281 so we supposed that a cannabinoid mechanism might not be involved in producing the analgesic effect of high doses of metamizole sodium in hot plate test. This statement is in accordance with some data from the literature which denies a cannabinoid mechanism for metamizole sodium [11].

The antipyretic effect of metamizole sodium is well-known. The question is if this substance might also have a hypothermic effect. A hypothermic effect was described in a patient with malignant lymphoma who received metamizole sodium [3]. In previous tests carried out in our laboratory, we obtained an important hypothermic effect for 1000mg/kg bw of metamizole sodium, with an intensity statistically comparable with the hypothermic effect of 10 mg/kg bw of chlorpromazine. This effect was registered at 30 minutes after dugs administration and lasted more than 2 hours, but its intensity decreased in time (at 2 hours it was less intense than after 30 minutes) [7].

In test 2 an important hypothermic effect was obtained for the groups treated with metamizole sodium 1000mg/kg bw alone or associated with AM 281 2mg/kg bw (without a statistical difference between the two groups). The hypothermic effect of metamizole sodium was not at all reduced by the association of AM281. This observation might signify that the hypothermic mechanism of metamizole sodium does not involve CB1 cannabinoid receptors.

The previous results from test 1 and test 2 denied an agonist effect on CB1 receptors for metamizole sodium.

There were also described as producing the tetrad some neuroleptics (including chlorpromazine). Another possible mechanism of action for metamizole sodium in the tetrad was investigated. Out of the four components of tetrad, catalepsy was chosen for underlying mechanism evaluation. The possible blockade of dopaminergic receptors, like for chlorpromazine, might be this mechanism. A dopaminergic agonist, bromocriptine, was administered in order to evaluate if this substance could antagonize the cataleptic effect of metamizole sodium or chlorpromazine. The results of test 3 showed that bromocriptine (4 mg/kg bw, 2 administrations in 24 hours) blocked the cataleptic effect of chlorpromazine but not that of metamizole sodium. These results rule out the dopaminergic hypothesis, therefore blocking the dopaminergic receptors cannot be the mechanism of action of metamizole sodium.

We may conclude that including a certain substance in the cannabinoid profile in accordance with the tetrad is not a sufficient argument in order to be sure that the concerned substance interferes with the endogenous cannabinoid system. This affirmation is in accordance with the experimental results previously obtained [13].

Conclusions

The cannabinoid-like effects of metamizole sodium are not produced by the interaction with the endogenous cannabinoid system because they are not antagonized by specific blockage of CB1 receptors.

The cannabinoid like effects of metamizole sodium are not produced by the interaction with the dopaminergic system, as for chlorpromazine, another catalepsy inducing substance, because they are not antagonized by bromocriptine, a dopaminergic agonist.

The effects obtained for metamizole sodium in the tetrad in mice might be due to different mechanisms of action. Out of these effects the hypothermic one may have future therapeutic applications in controlled hypothermia in humans.

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Manuscript received: January 17th 2013