

EXPERIMENTAL DEMONSTRATION OF HYPERALGESIA INDUCED BY REPEATED INGESTION OF DIETARY MONOSODIUM GLUTAMATE

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Abstract

Hyperalgesia and allodynia are symptoms of serious pathologies such as neuropathic pain, diabetic neuropathy, rheumatoid arthritis and osteoarthritis. These symptoms depend, in part, on the stimulation of glutamatergic receptors, which activates glutamatergic signaling nociceptive pathways. Glutamic acid (as sodium salt) is regulated in the European Union as a flavouring agent (coded as E 621). Through repeated use, a food addictive behaviour develops, leading to the abuse of food containing E 621. In this study, we established if monosodium glutamate, after repeated oral administration, can activate and sensitize glutamatergic receptors in pain pathways, inducing hyperalgesia. Our experiments were performed on young adult NMRI mice, using the Hot Plate test (Ugo Basile). Our results showed significant changes in the thresholds of pain perception and reaction, even at monosodium glutamate (MSG) dose levels matching those the humans receive as additive, through dietary intake.

Rezumat

Hiperalgezia și alodinia sunt simptome ale unor patologii severe precum durerea neuropată, neuropatia diabetică, artrita reumatoidă și osteoartrita. Aceste simptome sunt parțial datorate activării receptorilor glutamatergici și, implicit, intensificării semnalizării glutamatergice la nivelul căilor nociceptive.

Acidul glutamic, sub formă de sare sodică, este utilizat în Uniunea Europeană ca potențiator de aromă (notat E621). Datorită utilizării frecvente, sarea monosodică a acidului glutamic prezintă potențial adictiv, ceea ce determină abuzul alimentelor conținând acest aditiv alimentar. În acest studiu, am dorit să investigăm dacă administrarea orală repetată a glutamatului monosodic poate activa și sensibiliza receptorii glutamatergici la nivelul căilor durerii, inducând hiperalgezie. În scopul acestui experiment s-au utilizat șoareci albi, adulți NMRI, în cadrul testului *hot-plate*. Rezultatele noastre au semnalat modificări semnificative ale comportamentului animalelor la durere, chiar și la doze de glutamat monosodic similare celor primite de om prin alimentație.

Keywords: monosodium glutamate (MSG, E 621), hyperalgesia, hot-plate.

Introduction

Monosodium glutamate (MSG), the salt of glutamic acid (GLU), is largely used as food additive (E621). Two enantiomers are known, but only form L, which is the one naturally occurring, is used as flavour enhancer [1].

MSG activates *chorda tympani* (CT) neurons, by stimulating separate receptors for Na⁺, sugars and glutamate in taste bud cells [2]. The specific savoury taste („umami”) is elicited by both components of MSG: glutamate anion and Na⁺ cation [3]. Literature mentions that the GLU intake from food additives shows broad variations: high consumers in Europe may reach up to 1 g/day, whereas in Asian countries 4 g/day [1]. Our previous research demonstrates an addictive behaviour induced by MSG dissolved in drinking water, when given to mice (unpublished data). Single and double blind clinical studies with MSG reported: gastric discomfort, stiffness and a dose-dependent sensation of pressure, heat or burn which at high doses led to pain [4]. Also, GLU represents the main central excitatory neuromediator, involved in fast synaptic transmission and in long-term structural remodelling of the brain, associated with learning and memory (neuroplasticity). An imbalance in GLU homeostasis has been correlated with psychopathological disorders [5, 6].

GLU plays an essential role in nociception [7, 8]. It is located in 90% of peripheral nervous fibres, next to P substance [9]. GLU activates specific receptors: NMDA, AMPA and mGlu, located in central and peripheral pain pathways [10, 11]. The neural sensitization involving glutamatergic signaling is associated with neuropathic pain due to damage of central nerves (spinal cord lesions) or peripheral nerves (diabetic neuropathy) and joint pain (rheumatoid arthritis, osteoarthritis). The associated symptoms include hyperalgesia (increased sensitivity to pain) and allodynia (reduction of nociceptive threshold leading to perception of pain at non-painful stimuli) [12]. In consequence, NMDA antagonists (ketamine, amantadine, memantine, dextrometorphan) are used for the treatment of neuropathic pain and their spinal administration inhibits wind-up effect. Mixed μ -agonist and NMDA-antagonists (methadone, pethidine) represent the first-line therapy for prevention of opioid induced hyperalgesia and tolerance [13].

Given this data, we tried to experimentally prove the hypothesis that GLU when administered orally, as MSG, can activate and sensitize glutamatergic receptors in pain pathways inducing hyperalgesia in healthy mice.

Materials and Methods

Materials

Monosodium Glutamate (MSG = E 621) was purchased from Sigma-Aldrich.

Hot Plate (model 7280, Ugo Basile, Italy).

Experimental animals

90 NMRI adult mice, weighing 25 ± 2 g, were purchased from biobase of the *Carol Davila* University of Medicine and Pharmacy, Bucharest, Romania. They were housed in an individualized ventilated cage system, with free access to water and granulated food. The temperature ranged between 20 - 22°C and the relative humidity was maintained at 35-45%.

All procedures were carried out in accordance with the Directive 86/609/EEC-24th Nov.1986 and RGO 37/30.01.2002, on the protection of animals used for experimental and other scientific purposes.

Methods

The pain perception (53°C) and the pain reaction (53°C, 56°C) thresholds were assessed using the Hot-Plate test [14]. The animals were placed on the heated hot-plate (53°C or 56°C). The latency of the first sign of pain (hind paw lifting) and the latency of jumping response were assessed. In absence of a response, the cut-off time was set at 45 sec. Jumping was defined as both hind paws coming off of the hot plate while the front paws were already off. Hind paw lifting was defined as lifting a hind paw completely off of the hot plate in a way that was in no way related to mouthing, jumping, or taking a step.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. The type of distribution of the animal response was established with D'Agostino & Pearson test. The statistical analysis used parametrical Student *t* test (for normal distribution) or nonparametrical Wilcoxon test (for abnormal distribution). The results were expressed as mean \pm standard deviation.

Experimental protocol

The mice were divided in 6 working groups of 15 mice each. Each group was submitted to the regimen described below.

Table I
Experimental protocol – dosage and testing regimen

Group no.					
IA	IB	IIA	IIB	IIIA	IIIB
(control group/53°C)	(control group/56°C)				
The groups received for 21 days the following substances:					
Distilled water 0.1 mL/10g b.w. p.o.		MSG 100 mg/kg b.w. (solution 1%) p.o.		MSG 375 mg/kg b.w., (solution 3.75%) p.o.	
All animals were submitted to hot plate test in day 1 before exposure to MSG, and after 21 days of exposure, as it follows:					
Hot Plate temperature =53°C	Hot Plate temperature =56°C	Hot Plate temperature =53°C	Hot Plate temperature =56°C	Hot Plate temperature =53°C	Hot Plate temperature =56°C
The parameters recorded were :					
- latency of the first sign of pain (for Hot Plate temperature =53°C);					
- latency of jumping response (for Hot Plate temperature =53°C, =56°C).					

Taking account the LD₅₀ for mice (15000 mg/kg b.w.), the chosen doses were 375 mg/kg b.w. (equals 1/40 LD₅₀) and 100 mg/kg b.w. (under 1/100 LD₅₀). Preliminary research established that a period of administration of 21 days is necessary for inducing hyperalgesia. The withdrawal of one posterior paw from the hot-plate was considered the first sign of pain.

Results and Discussion

Hot Plate Test – temperature of 53°C

At a hot plate temperature of 53°C, the latency of the first sign of pain was recorded for groups IA, IIA, IIIA. The results are presented in table II.

Table II

Latency of the first sign of pain determined in day 1, before exposure (initial=I), and after 21 days of exposure (post-exposure=Pe) to MSG for groups IA, IIA, IIIA

Time of the first sign of pain at 53°C						
Group	I A		II A		III A	
	Initial	Post-exposure	Initial	Post-exposure	Initial	Post-exposure
Mean±	12.72±	10.74±	12.05±	8.59±	12.19±	7.26±
DS	7.06	4.13	5.31	4.40	6.12	3.00
Normal distribution	Yes	Yes	Yes	Yes	Yes	Yes
Δ % from initial value (Pe-I)*100/I		-15.57		-28.71		-40.43
Statistical significance (p<0.05)		Ns p=0.2094		** p=0.0056		* p=0.0156

DS – standard deviation; Δ – percental change; * - statistically significant ** - with high statistical significance, *** - with extremely high statistical significance, ns – with no statistical significance

A significant decrease of latency of the first sign of pain when compared with the initial value was seen only for groups receiving MSG solution (-28.71%, for group IIA dose level 100 mg/kg, respectively -40.43% for group IIIA dose level 375 mg/kg).

When compared to the control group, the decrease in the latency of the first sign of pain (reduction of pain perception threshold) is significant only for group IIIA, as illustrated in figure 1.

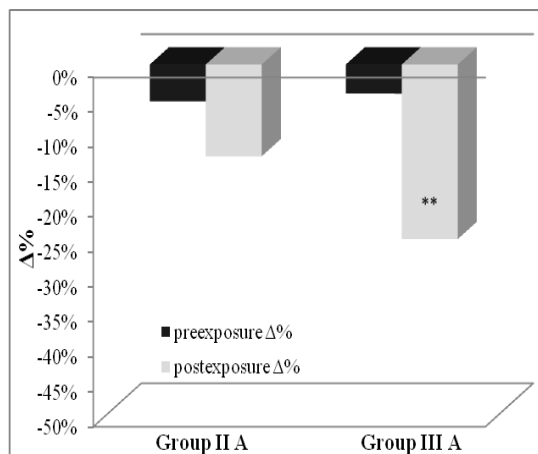


Figure 1

Percentual variation of latency of the first sign of pain at 53°C, for groups IIA and IIIA compared to control group, before and after 21 days exposure to MSG
 $\Delta \%$ vs. control of glutamate group = $\Delta \%$ vs. initial value of glutamate group - $\Delta \%$ vs. initial value of glutamate group

At a hot plate temperature of 53°C, the jumping time was also recorded for groups IA, IIA, IIIA. The results are presented in table III.

Table III

The latency of jumping response determined in day 1, before exposure (initial=I), and after 21 days of exposure (post-exposure=Pe) to MSG for groups IA, IIA, IIIA

Jumping time at 53°C						
Group	I A		II A		III A	
	Initial	Post-exposure	Initial	Post-exposure	Initial	Post-exposure
Mean±	42.47±	35.87±	43.61±	33.89±	43.34±	23.59±
DS	4.83	10.51	3.26	10.83	3.98	13.78
Normal distribution	No	Yes	Yes	No	No	Yes
$\Delta \%$ from initial value (Pe-I)*100/I		-15.53%		-22.30%		-45.56%
Statistical significance (p<0.05)		Ns p=0.0547		** p=0.002		*** p=0.0005

DS – standard deviation; Δ – percentual change; * - statistically significant, ** - with high statistical significance, *** - with extremely high statistical significance, ns – with no statistical significance

A significant decrease in the latency of jumping time when compared to initial values, is observed for both groups receiving MSG solution (-22,30%, for group IIA, respectively -45,56% for group IIIA).

When compared to the control group, a decrease of jumping time (reduction of pain reaction threshold), is significant only for group IIIA, as illustrated in figure 2.

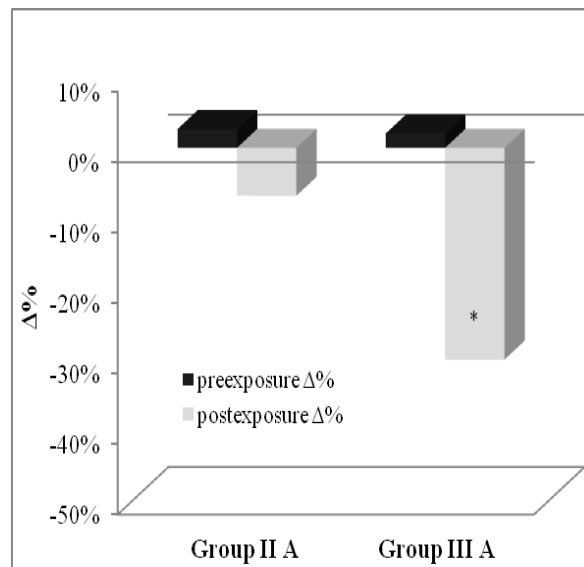


Figure 2.

Percentual variation of latency of the jumping response at 53°C, for groups IIA and IIIA compared to control group, before and after 21 days exposure to MSG
 $\Delta\%$ vs. control of glutamate group = $\Delta\%$ vs. initial value of glutamate group - $\Delta\%$ vs. initial value of glutamate group

An increase in the number of animals jumping at a temperature of 53°C, significant for group IIIA, when compared to control group, was observed, as illustrated in figure 3.

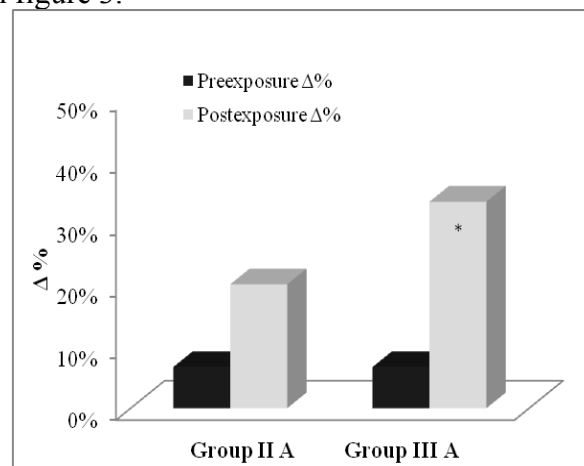


Figure 3.

Percentual variation of mice jumping at 53°C, for groups IIA and IIIA compared to control group, before and after 21 days exposure to MSG

Hot Plate Test – temperature 56°C

At 56°C, we recorded only the jumping time for groups IB, IIB, IIIB. The results are presented in table IV.

Table IV

Latency of jumping response, determined in day 1, before exposure (initial=I), and after 21 days of exposure (post-exposure=Pe) to MSG, for groups IB, IIB, IIIB

Jumping time at 56°C						
Group	I B		II B		III B	
	Initial	Post-exposure	Initial	Post-exposure	Initial	Post-exposure
Mean±	38.63±	22.27±	39.06±	16.97±	39.52±	13.78±
DS	6.80	11.59	7.32	14.28	7.98	12.32
Normal distribution	No	Yes	Yes	Yes	No	No
Δ % from initial value (Pe-I)*100/I		-42.34		-56.55		-65.13
Statistical significance (p<0.05)		*** p=0.0002		*** p< 0.0001		*** p=0.0001

DS – standard deviation; Δ – percentual change; * - statistically significant , ** - with high statistical significance , *** - with extremely high statistical significance , ns – with no statistical significance

A significant decrease in the latency of jumping response, when compared to the initial value, is seen for all groups, including the control, at 56°C. Nevertheless, when compared to control group (IB), only the decrease noticed for group IIIB (-22,79%) had statistical significance, as illustrated in figure 4.

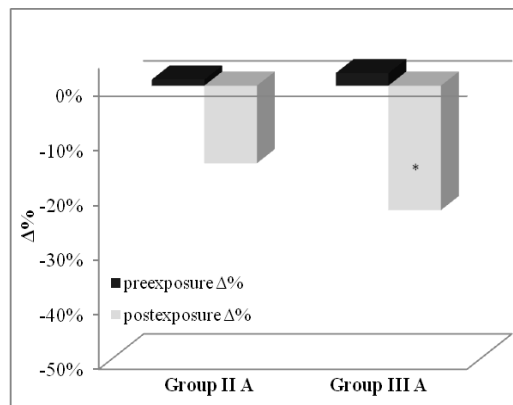


Figure 4

Percent change of latency of jumping response at 56°C, for groups IIB and IIIB compared to control group, before and after 21 days exposure to MSG
 Δ % vs. control of glutamate group= Δ % vs. initial value of glutamate group - Δ% vs. initial value of glutamate group

Due to the fact that jumping represents the first sign of pain for some mice and, also, that the variation between final and initial latency for the control group has extremely high statistical significance, the temperature of 56⁰C should not be considered in further research.

Our results showed that the administration of monosodium glutamate for 21 days, reduces the thresholds for both pain perception and pain reaction. Our results are in accordance with literature data supporting the involvement of glutamatergic signaling in neural sensitization which is one of the underlying mechanisms of neuropathic pain [15]. The activation of glutamate receptors plays a key role in the development of hyperalgesic responses due to pain of various etiologies [16-18]. Furthermore, we suggest that one of the possible mechanisms involved in the hyperalgesia induced by the administration of MSG, could be the activation of AMPA and NMDA receptors which have been linked with wind-up generation (a progressive increase in the number of action potentials generated by dorsal cord neurons, leading to pain sensitization) [19, 20].

Conclusions

The results sustain the hypothesis that, when administered in healthy experimental animals, MSG can induce hyperalgesia.

At 53⁰C, a significant decrease in the latency of the first sign of pain and in the latency of jumping response was seen for both groups receiving MSG, when compared to initial values. When compared to control group, this decrease was significant only for group III A (-24.86%, respectively -30.04%). When compared to control group, a significant increase in the number of animals jumping at 53⁰C (33.36%), was also observed for group IIIA.

At 56⁰C, a significant decrease in the latency of the jumping response, when compared to control, was noticed for group IIIB (-22.79%).

MSG influences both thresholds of pain perception and reaction, even at dose levels matching those humans receive through dietary intake. This raises questions on the extensive use of GLU or its salts as flavour enhancers in various processed aliments.

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