Evaluation of the antinociceptive potential of sodium valproate in mice

Evaluarea potenţialului antinociceptiv a valproatului de sodiu la şoarece

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Abstract

Recent research in the field of algesiology demonstrated the existence of several types of pain, with complex mediation cascades. This fact prompted the use of compounds from other drug families than classical analgesics for the treatment of pain. The purpose of this paper is to demonstrate the antinociceptive action of sodium valproate in mice. This investigation relies on three nociception models that use chemical, thermal, mechanical (pressure) stimuli, and a model of acute inflammation induced by carrageenan. The investigations were made using male white Swiss mice, weighing 20-30 grams. Valproate administration has been made orally, using geometric progression dose sequences. According to the statistical analysis, we obtained values of ED_{50} for sodium valproate for each nociception model as follows: ED_{50} = 21.773 ± 6.786 mg/kg for the nociception model with Zymosan A, ED_{50} = 11.807 ± 4.035 mg/kg, for the hot plate test, ED_{50} = 19.247 ± 2.207 mg/kg for the Randall-Sellitto test. The action of the valproate is explained by the inhibition of pro-inflammatory cytokines like TNF-α, IL1β, IL6 and prostaglandin mediators like PGE2. The experiments were made according to the European and Romanian legislation that concerns working with lab animals.

Rezumat

Cercetările din ultimii ani în domeniul algeziologiei au demonstrat existenţa mai multor tipuri de durere cu mediaţie complexă ceea ce a atras utilizarea unor compuşi din alte clase decat analgezicele clasice pentru tratamentul durerii. Scopul acestei lucrări este de a demonstra acţiunea antinociceptivă la şoarece a valproatului de sodiu. În prezenta lucrare se utilizează trei modele de nocicepţie care utilizează stimul chimic, termic şi presiune şi un model de inflamaţie acută indusă prin carrageenan. S-a lucrat pe şoareci albi masculi, Swiss cu greutăţi cuprinse între 20-30 g. Administrarea valproatului s-a efectuat pe cale orală prin administrarea unor secvenţe de doze în progresie geometrică. În urma analizei statistice s-au obţinut valori DE50 ale valproatului de sodiu pentru fiecare model de nocicepţie după cum urmează: DE_{50} = 21.773 ± 6.786 mg/kg pentru modelul de nocicepţie cu Zymosan A, DE_{50} =11.807 ± 4.035 mg/kg, pentru testul Hot plate, DE_{50} = 19.247 ± 2.207 mg/kg pentru testul Randall-Sellitto. Acţiunea valproatului se explică prin inhibarea citokinelor proinflamatoare de tip TNf - alfa, IL1, beta, IL6 şi a mediatorilor de tip PGE2. Experimentele s-au efectuat în accord cu legislaţia în vigoare în ceea ce priveşte lucrul cu animalele de laborator.

Introduction

During the last years, pain has been considered not only a symptom, but also a disease in itself. The investigations in the field of algesiology became extensive and brought to attention the existence of several types of pain, with a complex mediation.

Knowing the mediators involved, the researchers sought new therapeutic possibilities in the treatment of pain.

Drug from classes other than opioid analgesics and non-opioid or non-steroidal anti-inflammatories (NSAIDs) have proven their analgesic action isolated (tricyclic antidepressants, anticonvulsants), or in

combination with other substances. A number of anticonvulsants such as carbamazepine and valproic acid are useful in the trigeminal neuralgia and neuropathic pain [17].

In this paper, we are attempting to demonstrate the antinociceptive action of sodium valproate in mice, using a number of different models of nociception involving various mediation chains.

1. Materials and Methods

The study was conducted on male Swiss mice (Source Bucharest Cantacuzino Institute) weighing 20-30 g. The animals were placed in plexiglas cages provided with water dripper.

The habitation conditions were set inside the experimental Pharmacodynamics laboratory in the department of Pharmacodynamics and Clinical Pharmacy, UMF "Grigore T. Popa" Iasi, in a room with controlled temperature and humidity (21°C ± 2°C) and a cycle of light/dark, 12 / 12 hours (07.00 AM/07.00PM).

The animals received standard food and water ad libitum (Biobase Băneasa). The tests were carried out beginning with 10.00 AM and 3 hours before testing access to food and water was discontinued.

All experimental procedures used in this study were in close agreement with the specific approved norms of the "Grigore T. Popa" University of Medicine and the international bioethical regulations relating to experiments conducted on laboratory animals [18].

For demonstrating the antinociceptive action in this study was used:

- sodium valproate (Sigma),
- Zymosan A (Sigma),
- lambda carrageenan (Sigma),
- CMC-Na (Sigma) and
- saline (Zentiva).

The investigations into nociception were based on chemical, thermal, and mechanical stimuli.

The test of the abdominal constrictive response (methods of Siegmund et al. 1957, Koster et al., 1959) induced by Zymosan A, meaning the intraperitoneal administration of a suspension of Zymosan A at a dose of 40 $mgkgbw^{-1}$. The number of abdominal constriction responses for 12 minutes after administration of irritants is recorded [13].

Data interpretation of was quantal, characterized by the presence or absence of responses calculating the maximal possible effect:

% (antinociception) inhibition = (no. non-responders / total no. of animals) x 100 [11].

The hot plate test (early method of Woolfe and Mac Donald (1944), modified by Eddy and Laborit (1953), relies on the application of a thermal stimulus (52.5°C), during 30 seconds (*cutoff time*), on the plantar faces of the mouse's paws [4].

We used the Hot Plate 7280 Ugo Basile system, with thermostat for maintaining a constant temperature and measurement of the exposure time to the thermal stimulus. Data interpretation was of the gradual type. The antinociceptive effect was appreciated using the formula:

$$\% \text{ (antinociception) inhibition} = (T_x-T_0)/(T_m+T_0) \times 100,$$

Where: T_0 – the latency of the response measured before the administration of the study substance, T_x – latency after various time intervals following the administration of the substance, T_m – maximal allowed time (cut-off time).

The Randall-Selitto test allows the assessment of pain and inflammatory conditions and consists in the application of a mechanical stimulus (pressure) on the inflamed paw of the animal (cut-off pressure of 250g) [4].

The edema is produced by the subcutaneous injection into the plantar region of 3% saline suspension of carrageenan λ in mice. For the test was used the Ugo Basile analgesy-meter 37215. Data interpretation was of the graded type.

The antinociceptive effect was determined by the formula:

% (antinociception) inhibition = $(g_x+g_0)/(g_x-g_0)$ x 100

where: g_0 is the measured response latency before the administration of the substance, g_x - latency at different times in a row after the substance administration, g_m - the maximum permissible weight (cut-off weight).

The evaluation of the inflammatory edema was carried out by measuring the volume of the inflamed paw after subcutaneous administration in the plantar region of a saline suspension of 3% λ-carrageenan in mice [5, 13].

For this task, we used the Ugo Basile model 7200 plethysmometer. The inflammatory edema calculated the degree of inhibition, using the formula:

% inhibition = (M-T) / M x 100,

Where M = value of the degree of inhibition of the control group, T = value of the degree of inhibition in the treated group.

Statistical analysis of the data

In order to study the relationships between dose and effect, the work protocol requires the plotting and the analysis of regression lines for both types of effects.

For measuring the relationship between the two variables (the dose-effect), we used the Pearson correlation coefficient "R".

For assessing the antinociceptive potential, the Effective Dose 50 (ED_{50}) will be calculated, out of the maximum possible measured effect. In the statistical analysis for all tests, it has been considered that for P values < 0.05 there is a statistically significant difference between the groups compared (ANOVA test).

Results and Discussions

Via the oral administration of subsequent doses, ranging from 5.00-40.00 $mgkgbw^{-1}$, in geometric progression, of a suspension of sodium valproate in 0.1% CMC-Na was obtained for the abdominal constriction test a maximum possible antinociceptive effect (MPE) of 80.00%. (Table 1).

Table 1

Evaluation of the antinociceptive potential of sodium valproate

Nociception model	Sodium valproate $Mgkgbw^{-1}$ p.o	MPE (%)	Statistical parameters
Constrictive abdominal response test	40.00	80.00	ED50 = 21.773 ± 6.786 Eqn: Y = 1.943 + 2.285*X, R: 0.921 True Confidence Limits (11,338,115.64) log(1.055,2,063)
	20.00	50.32	
	10.00	33.33	
	5.00	0.00	
Hot plate test	20.0	81.32	ED50 = 11.807 ± 4.035 Eqn: Y = -29.880 + 74.504*X, R: 0.887 True Confidence Limits (11.685,2.169) log(1.068,0.336) ANOVA d.f.(1,2) F(calc): 17.379, F(tab): 18.51
	10.00	26.32	
	5.00	16.00	
	2.50	10.13	
Randall Sellitto test	40.00	65.4	ED50 = 19.247 ± 2.207 Eqn: Y = -30.126 + 62.386*X R: 0.982 True Confidence Limits (12.044, 42.916) log(1.081,1.633) ANOVA d.f.(1,2) F(calc): 52.776, F(tab): 18.51
	20.00	56.4	
	10.00	34.8	
	5.00	10.23	
Inflammatory paw edema	40.00	48.95	ED50 = 45.753 ± 6.964 Eqn: Y = -4.579 + 32.870*X , R: 0.989 True Confidence Limits (20.097,10.854) log(1.303,1.036) F(calc): 43.285, F(tab): 161.4
	20.00	36.45	
	10.00	29.16	

The data in Table 1 allowed plotting the regression curves for the determination of sodium valproate ED_{50} for all models of nociception studied (Figures 1 to 4).

From the regression analysis (Fig 1) and Table 1 it can be is observed that we could demonstrate the antinociceptive effect of sodium valproate (ED_{50} = 21.773 ± 6.786 $mgkgbw^{-1}$) in the nociception model with Zymosan A. The abdominal constriction response test allows for the assessment of central and peripheral analgesia.

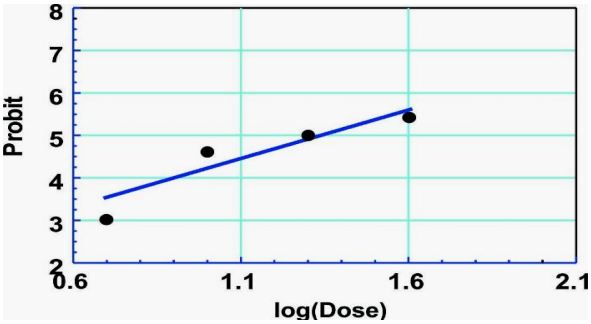

Fig. 1 The regression line for determining the ED_{50} for the valproate using the abdominal constriction test

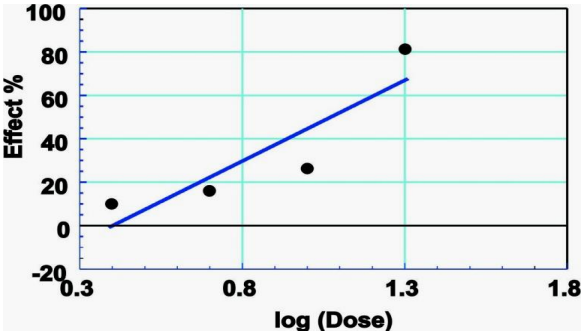

Fig. 2 The regression line for determining the ED_{50} for valproate using the hot-plate test

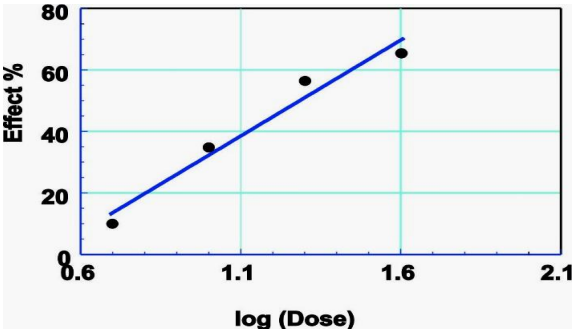

Fig. 3 The regression line for determining the ED_{50} for valproate using the Randall Selitto test

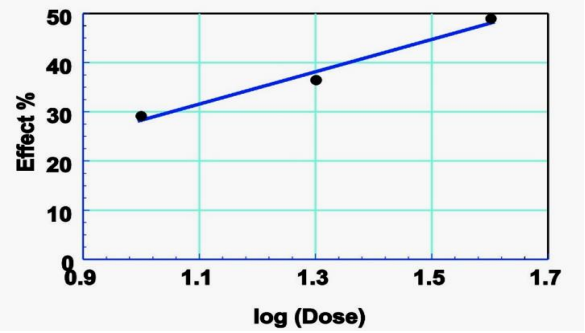

Fig. 4 The regression line for determining the ED_{50} for valproate using the inhibition degree of the inflammatory edema

A number of authors have shown that intraperitoneal administration of Zymosan in mice induces an inflammatory response, characterized by the abdominal constriction response, the extravasations of plasma, leukocyte infiltration and biosynthesis of eicosanoids (Doherty cit Pettipher, 1997) [7].

Unlike the abdominal constriction response produced by the intraperitoneal administration of acetic acid, the administration of Zymosan A does not produce cell necrosis, and therefore can be more relevant to the pathogenesis of inflammatory pain. [7, 14].

Recently it was revealed that inflammatory agents do not directly stimulate the release of primary hyper-nociceptive mediators, but that their release is preceded by a cascade of cytokines [8]. It was revealed that in mouse, the abdominal constriction response to Zymosan and acetic acid is mediated by TNF-α, IL1-β and IL8, which act simultaneously and synergistically (Ribiero cit Cunha 2005) [1].

The action mechanism of the valproic acid is the inhibition of the enzyme GABA-transaminase, responsible for the degradation of GABA and for the increase in the activity of the glutamic acid decarboxylase, acting indirectly on GABA [16].

The literature brings out also other possible mechanisms of action that may contribute to its antinociceptive effect (attenuation of NMDA excitatory action,

blocking of Na channels and of voltage-dependent calcium channels (type L, C, D, N, F, T) and voltage-dependent potassium channels [3, 9, 15].

Other studies show that valproic acid reduces the number of leukocytes and the release of myelo-peroxidase in the peritoneal exudates in the model of peritonitis with carrageenan that produces an inflammatory response with involving neutrophils infiltrate, plasma exudation, cell migration and release of mediators like NO, PGE2, IL 1 β, IL-6, TNF-α [10, 16].

From the regression analysis shown in Fig. 3 and from the data in Table 1 we can observe an antinociceptive effect of sodium valproate (ED_{50} = 19.247 +/- 2.207) also for the inflammatory nociception model induced by carrageenan.

Recent studies demonstrate the antinociceptive and anti-inflammatory effect of sodium valproate on the paw edema induced by carrageenan [16].

For the inhibition of the inflammatory oedema and the dose sequence of taken in study we could not identify an ED_{50} value (Table 1).

The ED_{50} value = 11.807 ± 4.035 $mgkgbw^{-1}$ (Table 1, Fig. 2) obtained for the hot plate test can be explained by the intervention of valproic acid on the Na channels from the DRG neurons, including the indirect inhibition of sub-threshold slow sodium currents and membrane-depolarizing rectification [12] and calcium currents [2].

Thus, a direct spin cortical inhibition may be surmised, because of the inhibitory effect on high-frequency action potential firing which is directly involved in pain perception [6]. To our knowledge, we are the first to present such an effect of valproic acid on a thermal acute pain model like the hot-plate.

Conclusions

Considering the common mediation for the two models of nociception (paw inflammatory edema induced by carrageenan and abdominal constriction response induced by Zymosan A), the close ED_{50} values and the resulting statistical parameters, we conclude that the inhibition of nociception in inflammatory pain caused by sodium valproate may be explained by the inhibition of the release of the pro-inflammatory cytokines TNF-α type, IL1-β, IL6 and PGE2.

We have also demonstrated a direct anti-nociceptive effect of sodium valproate on the pain perception using an acute thermal pain model.

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