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EXPERIMENTAL RESEARCHES ON THE EFFECTS OF A SELECTIVE KAPPA OPIOID AGONIST IN CUTANEOUS AND VISCERAL PAIN MODELS IN MICE

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Abstract. Aim. Experimental researches on the effects of a selective k opioid receptor agonist in cutaneous and visceral pain models in mice. **Material and method** The experiments were carried out on white Swiss mice (20-25g), divided into 4 groups of 7 animals each, treated intraperitoneally with the same volume of solution, as follows: Group I: distilled water (Control) 0,3ml; Group II (U-50488H 10): U-50488H 10mg/kbw; Group III (U-50488H 20): U-50488H 20mg/kbw; Group IV (MOR): morphine 2mg/kbw. Experimental protocols were implemented in accordance to the recommendations of the committee of research and ethics of the International Association for the Study of Pain. The nociceptive cutaneous testing was performed using the tail flick assay. The model of visceral pain consisted of inflammatory cystitis after intraperitoneal injection of cyclophosphamide (200 mg/kbw). The data were presented as +/- SD and significance was tested by SPSS for Windows version 13.0 and by the ANOVA method, followed by the Neumann Keuls test as post hoc. **Results and conclusions.** In our experimental conditions, U50,488H (10mg/kbw) determined antinociceptive significant effects in tail flick test, 30 minutes after thermal noxious stimulation, but did not influence visceral nociceptive responses in cyclophosphamide-induced cystitis. Intraperitonal administration of selective k opioid agonist U50,488H, 20mg/kbw, resulted in a potent analgesia in both cutaneous and visceral pain models.
Keywords: k opioid agonist, U50,488H, nociception, tail flick, cyclophosphamide.

Introduction:

Opioid receptors have been identified on peripheral sensory neurons of animals and humans. In addition to their efficacy in somatic pain, peripheral opioids potently inhibit visceral pain. Major recent findings in peripheral opioid analgesia include the relative lack of tolerance under inflammatory conditions, tetrapeptides as novel peripherally restricted compounds, the potent anti-inflammatory activity of μ and κ agonist derivatives. (Stein et al., 2003)

Many selective nonpeptide k opioid receptor agonists have been synthesized; most are arylacetamide compounds, including U50,488H, U69,593, ICI 204,448, and asimadoline. U-50488H, trans-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]benzeneacetamide methanesulfonate salt is a selective κ-opioid receptor agonist. Literature data shows that κ-opioids are of interest for the modulation especially of visceral pain.

The **aim** of our study was the experimental researches on the effects of different doses of a selective k opioid receptor agonist, in cutaneous and visceral pain models in mice.

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Material and method:

Substances: U-50488H (Sigma Chemical Co), morphine (Fiola, Bucureşti), cyclophosphamide (Sigma Chemical Co). The control solution was distilled water (Sicomed, Romania).

Animals: Male white Swiss mice (20-25g) were used. Standard laboratory food and tap water were freely available, except during the time of the experiments.

Procedure: The mice were distributed into 4 groups of 7 animals, treated intraperitoneally with the same volume of solution as follows: **Group I:** distilled water (Control) 0,3ml; **Group II** (U-50488H 10): U-50488H 10mg/kbw; **Group III** (U-50488H 20): U-50488H 20mg/kbw; **Group IV** (MOR): morphine 2mg/kbw. Morphine, subcutaneouslly administred, was used as a positive control substance in both experimental nociceptive models.

The nociceptive cutaneous testing was performed using the tail-flick assay. This experimental pain model consists of mice's tail thermal noxious stimulation followed by counting of the response latency period.

Intraperitoneal administration of antitumoral agent cyclophosphamide 200 mg/kbw modified the behavior of the mice with cystitis induced by acrolein, a toxic urinary byproduct of cyclophosphamide. Beginning approximately 1 hour after systemic administration and continuing for approximately 4 hours, unanesthetized mice demonstrated alterations in normal behavior. Hand-operated counters were employed for observing the behavioral manifestations of the mice placed in glass cages. (Olivar et al., 2000) (Boucher et al., 2000) These behavioral modifications (decreased breathing rate, closing of the eyes and specific postures) were scored for the assessment of nociception indirectly elicited by cystitis and for the use of this experimental model as vesical pain model. The first 2 hours postinjection cover a period of time over which inputs of multifactorial origin (stress and pain due to the intraperitoneal injection process) interact while the last 2 hours are more specific for inflammatory cystitis. (Westropp J.L., 2002) (Tarţău L., 2008) The mice were observed for 2 minutes every 30 min of 120-240 minutes time interval after cyclophosphamide treatment and their behavior was coded according to an arbitrary scale. If more than one of these behaviors was noted in one observation period, the sum of the corresponding points was assigned and it was calculated as behavior score. (Bon K. et al., 2003)

The experiment was performed according to the guidelines of the IASP Committee for Research and Ethical Issues. (Zimmermann, 1983) In particular, the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the experiment. The obtained results were analyzed with SPSS for Windows version 13.0. All thr values corresponding to each time period were expressed as arithmetic mean±SD and processed using ANOVA one-way analysis followed by Newmwn-Keuls post-hoc test. P- values under 0.05 are considered statistically significant compared with those of the control groups.

Results:

The results obtained in the tail flick test were presented in **figure 1**.

Statistical analysis of the results obtained in tail flick shows that:

- in our experimental conditions, k opioid

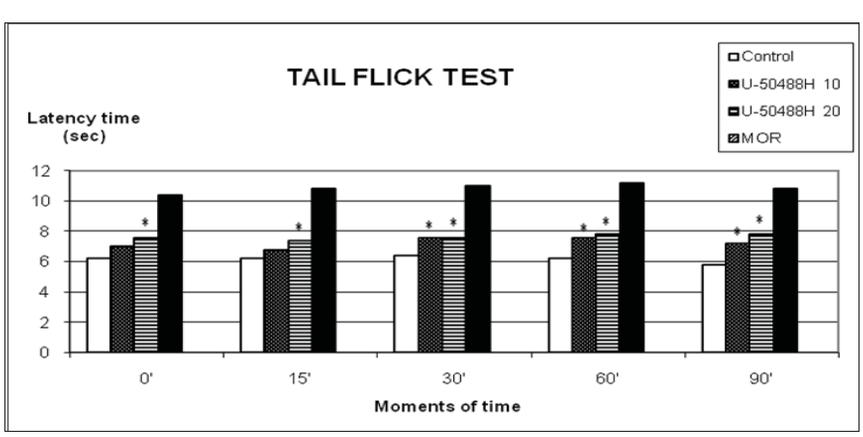

Figure 1. The effects of U-50488H in tail flick test (* p<0.05).

agonist U50,488H (10mg/kbw) decreased the latency time period of the response, 30 minutes after thermal noxious stimulation;
- intraperitoneal administration of a dose of 20 mg/kg U50,488H resulted in a significant ($p<0.05$) decrease of the latency time reaction, compared with the control group, but less intense than morphine with known analgesic effects in this test;

The results obtained in cyclophosphamide induced cystitis test were presented in **figure 2**.

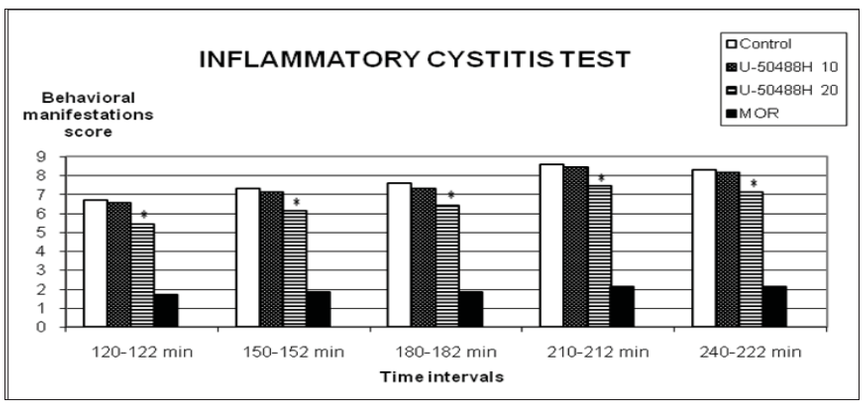

Figure 2. The effects of U-50488H in inflammatory cystitis test (* $p<0.05$).

Statistical analysis of the results obtained in cyclophosphamide induced cystitis test shows that:
- in our experimental conditions k opioid agonist U50,488H (10mg/kbw) did not influence the behavioral manifestations score;
- U50,488H (20mg/kbw) determined a significant ($p<0.05$) decrease of the behavioral manifestations score, compared with the control group, but less intense than morphine with known analgesic effects in this test;

Discussions:

Opioid receptors have been ascertained on sensory nerves in both inflamed and normal subcutaneous tissue but locally applied opioid agonists produce analgesia in inflamed tissue only. Inflammatory pain can be effectively decreased by activation of opioid receptors on peripheral terminals of sensory neurons. (Sawynok J., 2003) Selective activation of peripheral opioid receptors has the important advantage of providing effective analgesia without eliciting side effects typically associated with centrally acting opioids or with cyclooxygenase inhibitors (Stein et al., 2003)

Electro-physiologic studies show that results concerning the increased opioid agonist efficacy are due to an increased number of peripheral opioid receptors in later stages of inflammation and that peripheral opioid antinociceptive effects are primarily mediated by mu, delta and kappa opioid receptors on primary afferent neurons. (Zhou et al., 1998)

There are data that demonstrated the peripheral k opioid receptors' implication in antinociception mediation in localized inflammation upon injection of Freund's complete adjuvant into one rat hind paw. Local injection of kappa selective agonists [D-Ala2,N-methyl-Phe4,Gly-ol5]-enkephalin, [D-Pen2,5]-enkephalin and U-50, 488H produced marked antinociceptive effects in inflamed but not in noninflamed paws, results which suggest that several selective opioid agonists can modulate responses to noxious pressure through a peripheral opioid receptor-specific site of action in inflammatory conditions. (Stein et al., 1989)

Kappa opioid receptor agonist U-50488H also produced dose dependent antinociceptive effect in tail flick, in hot plate (Sternberg et al., 2004) (Broqua, 1998) (Dykstra et al., 1993) and in warm water tail withdrawal assays. (Craft et al., 2001)

Moreover, different other authors proved that U50,488-H dependently attenuated the writhing response dose (in an acid-induced visceral pain model writhing assay in mice) with complete inhibition occurring at the highest doses. (Gallantine et al., 2004) (Patrick et al., 1999)

Literature data shows that intrathecal or spinal administration of U50,488 had no effect on nociceptive responses in a model of chronic muscle

pain (determined by injections of acidic saline into one gastrocnemius muscle) respectively in different cutaneous and visceral pain models such as: tail-flick test (Przewlocka et al., 1991), hot plate test (Stevens et al., 1986), paw pressure test (Leighton et al., 1988), carrageenan paw inflammation test (Hylden et al., 1991) and colorectal distension model. (Danzebrink et al., 1995)

Conclusions:

- In our experimental conditions, U50,488H (10mg/kbw) determined antinociceptive significant effects in tail flick test, 30 minutes after thermal noxious stimulation, but did not influence visceral nociceptive responses in cyclophosphamide-induced cystitis.
- Intraperitonal administration of selective k opioid agonist U50,488H, 20mg/kbw, resulted in a potent analgesia in both cutaneous and visceral pain models.

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