

## Analgesic Activity of the Kappa Opioid Receptor Agonist - RU-1205 in Rats

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### ABSTRACT

**Introduction:** Opioid analgesics are the most efficient and widely used drugs for the management of moderate to severe pain. However, side effects associated with mu receptor activation, such as respiratory depression, tolerance and physical dependence severely limit their clinical application. Currently, the kappa-opioid system is the most attractive in terms of the clinical problem of pain, because kappa-agonists do not cause euphoria and physical dependence. The purpose of this study was to evaluate the antinociceptive effect of the novel compound - RU-1205. **Methods:** The analgesic activity of RU-1205 was studied on nociceptive models that characterize the central and peripheral pathways of pain sensitivity (hot plate test, electrically induced vocalisation, formalin test, writhing test). **Results:** RU-1205 exhibited highly potent antinociceptive effects in rodent models of acute pain with ED<sub>50</sub> values of 0.002 - 0.49 mg /kg. Pretreatment with the κ-opioid receptor antagonist norBinaltorphimine significantly attenuated the analgesic activity of investigated substance in a hot plate test. **Conclusions:** It was established that the compound shows a significant dose-dependent central and peripheral analgesic effect. It was assumed kappa-opioidergic mechanism of analgesic effect of RU-1205.

**KEYWORDS:** kappa-opioid agonist, opioid analgesics, butorphanol, U-50,488, norBinaltorphimine

## INTRODUCTION

Nowadays most analgesics administered in clinical practice produce a nonselective effect on opioid receptors [1]. However, μ-agonists produce a range of quite serious undesirable reactions. Respiratory depression is the most serious and dangerous side-effect resulting from opioid therapy [2]. Dependence and tolerance affect the outcome of narcotic analgesic therapy considerably, as well [3]. A prolonged consumption of opioids promotes the development of habituation to the analgesic effect, which eventually requires higher doses of the analgesic agent. The abuse potential of analgesic drugs may be the cause of difficulties in social adaptation once the therapy is over [1]. Therefore, development of opioid analgesics that do not cause side-effects typical of morphine-like drugs comes to the forefront [4]. Compounds showing kappa opioid receptor agonist activity can be regarded as substances that lack this undesired effect [5].

Preliminary experimental study on the directed search for substances with kappa-receptor profile of pharmacologic activity done at the Volgograd State Medical University resulted in isolating a new compound - RU-1205 that shows a pronounced kappa opioid effect [6]. Previously, we studied the antinociceptive effect of the substance on the spinal level of pain sensitivity – we estimate tail flick reflex. The objective of the present study was to explore the analgesic activity of RU-1205 by methods characterizing nociceptive response at the level of medulla oblongata (vocalization), peripheral pain (formalin test) and the mechanism of analgesia.

## METHODS

### Animals

Experiments were conducted on adult male rats (weight range 200-250 g) and mice (weight range 20-



25 g), 6-8 weeks of age. Animals were housed in group of 5 (mice) or 2-3 (rats) at a room temperature of  $20\pm 2^{\circ}\text{C}$  and under a 12-h light/dark cycle. Food and water were available ad libitum. The study protocol was approved by the local Ethics Committee of the Volgograd state medical university (No. 1351-KM of 12.11.2013), in accordance with the International Association for the Study of Pain guidelines on ethical standards for investigations of experimental pain in rodents. Furthermore, every effort was made to minimize animal suffering and the number of used animals was the minimal required.

## Drugs

The paper presents data from studying the analgesic activity and the mechanism of action produced by the substance of RU-1205 dihydrochloride 9-(2-morpholinoethyl)-2-(4-fluorophenyl)imidazo[1,2- $\alpha$ ]benzimidazole synthesized at the Research Institute for Physical and Organic Chemistry at Southern Federal University [7].

The experimental study was done using 2% formalin solution (Biomed, Russia), 0.6% acetic acid (Reachimservice, Russia), Butorphanol tartrate (Moskovskaya pharmatsevticheskaya fabrika joint stock company, Russia), U-50,488 selective kappa opioid receptor agonist (SIGMA, USA); naloxone, nonselective opioid receptor antagonist (Moskovskaya pharmatsevticheskaya fabrika joint stock company, Russia); norBinaltorphimine, selective kappa opioid receptor antagonist (norBNI) (SIGMA, USA).

## Injections

The compound and the standard drug- butorphanol tartrate, were dissolved in distilled water and administered intraperitoneally (i.p.) at a dose of 0.01, 0,1 and 1 mg/kg. Control animals received an equivalent amount of vehicle.

To evaluate the central aspect of nociception, we employed methods of thermal (hotplate test) and electric stimuli (electrical stimulation of the tail). The peripheral level of nociceptive sensitivity organization was evaluated using chemical stimuli: formalin test and acetic acid induced writhing test.

## Hot plate test

The hotplate test was performed with a modification of method described by Kitchen et al (1985) [8]. Fifty-six (n=8 in each group) male mice were included in the study and each mouse was tested only once. Mice were placed on the heated surface ( $55\pm 1^{\circ}\text{C}$ ) 60 min after the intraperitoneal injection of vehicle, RU-1205 or butorphanol. The latent period before first licking of hind paw was recorded in *second* as parameter for determining of nociception. To avoid injury, mice which did not respond within 30 s were removed from the hot plate and this value was taken as 100% inhibition of the nociceptive response.

## Electrical stimulation of the tail

Vocalization at the time of electrical stimulation was observed during gradually increasing intensities of electrical stimuli applied through subcutaneous electrodes on the rat's tail [9]. The method is based on measuring the threshold voltage at which nociceptive reflex – vocalization, appears. The animals – forty-eight adult male rats (n=6 in each group), were placed in horizontal aerated plastic cylinders and two electrodes were attached at the base of the tail. The electrodes were connected to a stimulator (ЭЦЛ-2, Russia) delivering the current [pulse frequency: 100 Hz, train duration: 100 ms, train interval: 1 s]. The thresholds for the vocalization during stimulus were assessed for each animal 60 min after the intraperitoneal injection of compounds. Increase of the voltage value when vocalization occur was interpreted as an analgesic action.

## Formalin test

This test was performed by the method of formalin-induced hyperalgesia with registration the first (0–10 min) and second phases (10–60 min) of pain reaction (number of flinches) after subcutaneously injection of 50  $\mu\text{l}$  2% formalin prepared into the rat's dorsal hind paw [10]. Forty-nine male rats (n=7 in each group) were used in this study and number of flinches was counted 60 min after the intraperitoneal injection of compounds. Reducing the number of flinches was regarded as an analgesic effect.

## Writhing test

The acetic acid-induced writhing test as a model of acute visceral pain was conducted according to the method described by Koster R. et al [6]. Forty-nine male mice (n=7 in each group) were used in this experiment. Mice were given an i.p. injection of 0.6% acetic acid solution (0.1 ml/10 g) 60 min after the administration of vehicle, RU-1205 or butorphanol, and the number of acid-induced writhes were counted for 15 min. Writhing included the following behaviors: contractions of the abdomen, twisting of the trunk, arching of the back, and extension of the hindlimbs.

## Tests with opioid antagonists

The nonselective opioid antagonist Naloxone and the selective kappa opioid receptor antagonist Norbinaltorphimine were used to estimate opioid activity of the studied compounds. Seventy male mice (n=7 in each group) were used in this experiment. RU-1205 compound and standard drug - selective kappa-opioid agonist U 50488, were injected intraperitoneally at a dose of ED<sub>80</sub> using regression analysis. RU-1205 exhibits highest activity on the central model of pain, therefore hot plate test has been selected in this study. Naloxone (10 mg/kg), norBinaltorphimine (10 mg/kg) were given subcutaneously (s.c.) 20 minutes prior to injection of compounds. Antinociceptive activity was calculated by the formula  $= (Lp_t - Lp_c) / (30 - Lp_c) \times 100\%$ , where Lp<sub>t</sub> – latent period in test compound, Lp<sub>c</sub> - latent period in control group, 30 – 30 sec maximal time on the plate.

## Data analysis

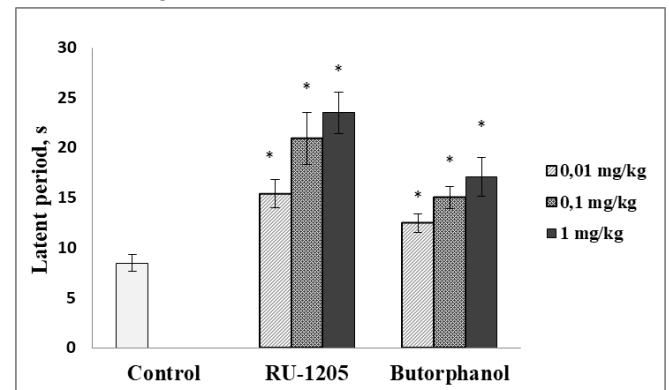
Data were expressed as mean  $\pm$  S.E.M. The data were analysed by Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA) followed by Mann-Whitney's U- test, using the GraphPadPrismVersion-5.0 software. P<0.05 was considered significant.

## RESULTS

### Hot plate test

The hot plate test revealed a dose-dependent antinociceptive activity of compound RU-1205. At a dose of 1 mg/kg, 0.1 mg/kg and 0.01 mg/kg the studied compound showed a significant increase in

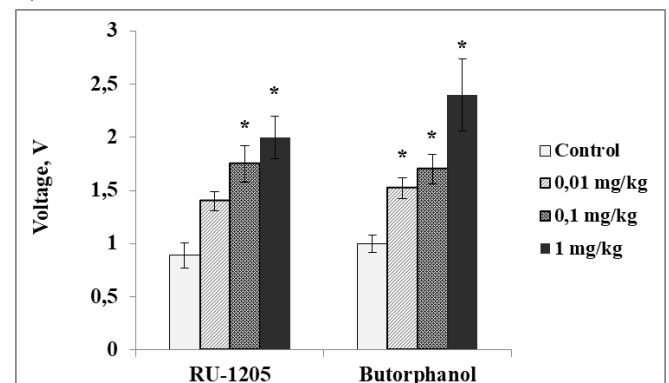
pain threshold in comparison with the controls: 2.8 times, 2.5 times and 1.8 times, respectively, while butorphanol tartrate at the same doses increased the latent period of nociceptive reaction 2, 1.8, and 1.5 times, respectively. No significant difference between the studied compound and the standard drug was revealed (Figure 1).



**Figure 1** Effect of RU-1205 compound and Butorphanol on pain threshold in hot plate test. \* - Significant difference compared with control ( $p \leq 0.05$ )

### Electrical stimulation of the tail

Gradually increasing electric stimulation of rat tail induced a nociceptive reaction of vocalization type. Upon intraperitoneal administration at a dose of 0.01, 0.1 and 1 mg/kg, compound RU-1205 induced significant pain sensitivity compared with the control; its activity was comparable with butorphanol (Figure 2).

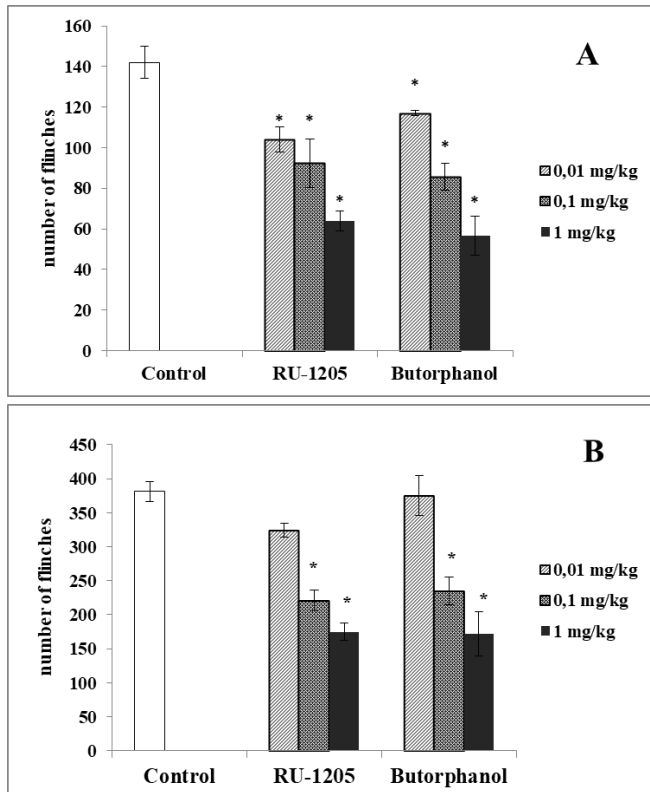


**Figure 2** Effect of compound RU-1205 and Butorphanol on pain sensitivity in vocalization test with electric stimuli. \* - Significant difference compared with control ( $p \leq 0.05$ )

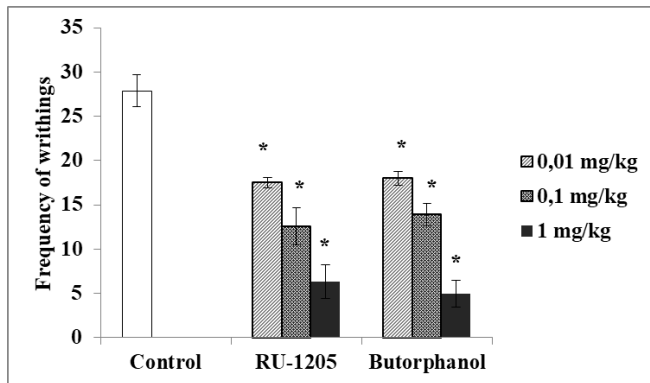
### Formalin test

A subcutaneous injection of formalin to controls induced pain reaction in the form of flinches. Numbers of flinching responses in the first phase was  $142.2 \pm 8.9$ ; in the second phase –  $381.6 \pm 15.2$ . In the first phase of formalin test compound RU-1205 injected intraperitoneally at a dose of 0.01, 0.1 and 1 mg/kg

decreased the number of flinches by 27%, 39% and 55%, respectively. In the second phase – by 15%, 42% and 54%, respectively. In these tests the studied compound showed an analgesic activity equal to that of standard drug Butorphanol (Figure 3).



**Figure 3** Effect of RU-1205 compound and Butorphanol on pain sensitivity in formalin test in the acute phase (A) and in the inflammation phase (B). \* - Significant difference compared with control ( $p \leq 0.05$ )



**Figure 4** Effect of compound RU-1205 and Butorphanol on nociceptive reactions in mice under the impact of chemical stimuli in acetic acid induced writhing test. \* - Significant difference compared with control ( $p \leq 0.05$ )

### Writhing test

Administered intraperitoneally, acetic acid irritates the serous membranes and induces contraction of abdominal musculature in mice. In the control group, the average number of writhing episodes was  $27.9 \pm 1.8$ . Compound RU-1205 caused changes in pain sensitivity: it induced a dose-dependent decrease in the

frequency of writhings. Thus, at a dose of 0.01 mg/kg the studied compound decreased the frequency of writhings by 37% compared with the controls; at a dose of 0.1 mg/kg and 1 mg/kg – by 55% and 77%, respectively. At a dose of 0.01 mg/kg, 0.1 mg/kg and 1 mg/kg Butorphanol, inhibited acetic acid induced writhings by 35%, 50% and 82%, respectively (Figure 4). On the basis of the data obtained, we worked out the median effective dose ( $ED_{50}$ ) for the studied substances (Table 1) using regression analysis.

**Table 1** Value of analgesic activity ( $ED_{50}$ ) and 95% confidence limits (in parentheses) for compound RU-1205 and Butorphanol on central and peripheral models of pain sensitivity

Test	Substance	$ED_{50}$
Central pathway of pain sensitivity organization		
Hot plate	RU-1205	0.002 (0.003-0.001)
	Butorphanol	0.006 (0.013-0.003)
Vocalization	RU-1205	0.003 (0.005-0.002)
	Butorphanol	0.01 (0.126-0.005)
Peripheral pathway of pain sensitivity organization		
Formalin test	Phase I	
	RU-1205	0.49 (0.67-0.36)
	Butorphanol	0.32 (0.34-0.30)
	Phase II	
RU-1205	0.43 (0.88-0.21)	
Butorphanol	0.48 (0.99-0.23)	
Acetic acid induced writhing	RU-1205	0.05 (0.06-0.04)
	Butorphanol	0.06 (0.11-0.03)

To confirm the opioid mechanism of action as well as the effect of the studied compound on the kappa receptor component of analgesic activity, we performed studies with Naloxone, nonselective opioid receptor antagonist and norBinaltorphimine, selective kappa opioid receptor antagonist in a hot plate test.

Upon preliminary subcutaneous injection of Naloxone, opioid receptor antagonist, at a dose of 10 mg/kg, the analgesic activity of compound RU-1205 and Butorphanol showed a significant decrease of 6.3 and 4.3 times, respectively (Table 2). A preliminary subcutaneous injection of norBinaltorphimine at a dose of 10 mg/kg induced a significant decrease of analgesic activity of compound RU-1205 and U-50,488 that was 6.2 and 2.2 times less, respectively (Table 3).

**Table 2** Effect of compound RU-1205 and Butorphanol tartrate on intact animals and those who received Naloxone in hot plate test

Group of animals	Latent period, s	Antinociceptive activity, %
Control (n=7)	7.5±0.4	
RU-1205 (n=7)	25.8± 1.7*	81.5%
Naloxone + RU-1205 (n=7)	10.4±0.8	12.9%
Butorphanol (n=7)	24.7±2.1 *	76.5%
Naloxone + Butorphanol (n=7)	11.46±1.1	17.6%

\* - Significant difference compared with control ( $p \leq 0.05$ )**Table 3** Effect of compound RU-1205 and butorphanol tartrate on intact animals and those who received norbinaltorphimine in hot plate test

Group of animals	Latent period, s	Antinociceptive activity, %
Control (n=7)	6.8±0.8	
RU-1205 (n=7)	28.5± 1.6*	93.8%
norBinaltorphimine + RU-1205 (n=7)	10.3±1.1	15.2%
U-50,488 (n=7)	26.5±2.2*	85.0%
norBinaltorphimine + U-50,488 (n=7)	15.8±2.8	39.2%

\* - Significant difference compared with control ( $p \leq 0.05$ )

## DISCUSSION

The most clinically applied opioid analgesics act as agonists at the mu-opioid receptor, but their non-medical purposes and adverse effects impede clinical usefulness and substantiate the immense effort put forth by numerous researchers to reveal safer opioid drugs or non-addictive analgesics. Kappa-selective agonists represent the promising group of substances for elaboration of opioid analgesics with original mechanism of action without the risk of respiratory depression and drug dependence [4].

Currently, there is strong evidence of the potential role of kappa-opioid receptor system in the development of analgesia, involving various structures of the cerebral cortex, middle, brain stem, spinal cord, peripheral nervous system, either of which contributes to the formation of nociception. Kappa receptors are expressed at different levels of perception of nociceptive information. Cells expressing mRNA for the kappa-receptor proteins with a high density detected in the amygdala, nucleus accumbens, basal nuclei, medial, reticular nuclei of the thalamus, supraopticus, paraventricular, dorsomedial,

ventromedial and lateral nuclei of the hypothalamus. Kappa-opioid receptors are highly concentrated in outer laminae of the dorsal horn of the superficial layers of the lumbosacral spinal cord and C-fibers of primary afferent neurons on the periphery [12].

Direct screening novel heterocyclic nitrogen containing derivatives performed at the Volgograd State Medical University in vitro and in vivo assays a series of novel chemical substances with high kappa-opioid agonist activity has revealed. The most perspective compound, novel benzodiazepine derivative - showed efficacious kappa-agonist activity using in vitro functional assays on rabbit isolated vas deferens preparations which express kappa-opioid receptors exclusively [7]. These facts was a prerequisite to study the analgesic activity of a new derivative of imidazo[1,2- $\alpha$ ]benzimidazole – RU-1205.

The nociceptive tests allow the assessment of different nociceptive reactions that appear to be associated to pain regulating systems existing at distinct levels of the central nervous system. Experiments on various nociceptive models revealed that compound RU-1205 has shown a dose-dependent analgesic effect in all tests. The central models with thermal and electric stimuli revealed that the studied compound RU-1205 has shown a pronounced analgesic activity in hot plate test and vocalization test based on behavioral reactions controlled by supraspinal structures, which suggests that compound RU-1205 produces an analgesic effect on supraspinal level. In the implementation of nociceptive responses in the "vocalization" tests, mainly involved the structures of the brain stem, whereas in the "hot plate" test cortical, subcortical neuronal formations form complex highly integrated protective reactions to thermal stimuli.

To date, there is convincing evidence that the kappa opioid receptor system plays a role in analgesia development involving peripheral pathways of the nervous system [13]. These facts warranted our evaluation of antinociceptive properties of compound RU-1205 on peripheral models of pain sensitivity in formalin test and acetic acid induced writhing test. It was established that the studied compound has shown a significant decrease in the frequency of writhings and flinches induced by chemical stimuli; its

antinociceptive activity is comparable to that of Butorphanol. The obtained findings make it possible to suppose that the effect on the peripheral link in pain reaction produced by the studied compound is an important issue, along with the central analgesic effect.

The obtained results are consistent with the literature data indicating the existence of peripherally-regulated kappa-opioid antinociception on models of somatic and visceral pain, especially under conditions of inflammation [5]. The analgesic effect shown by compound RU-1205 was blocked both by Naloxone, a nonselective opioid receptor antagonist, and norBinaltorphimine, a selective kappa opioid receptor antagonist. It was assumed that the studied compound shows antinociceptive properties that are implemented through kappa-opioidergic receptor mechanisms, which was detected by norBinaltorphimine tests.

## CONCLUSION

RU-1205 shows a significant analgesic effect on central and peripheral models of pain with most pronounced activity in the central models of nociceptions characterizing the supraspinal level of pain sensitivity (hot plate test), where the RU-1205 compound exceeded butorphanol by level of analgesic activity 3 times. Lessening of analgesic effect of RU-1205 by norBinaltorphimine tests was assumed kappa-opioidergic mechanism of action.

## Conflict of Interest

Authors declare none.

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