Research Results in Pharmacology

Research Results in Pharmacology 6(3): 59–65
UDC: 615.212.7
DOI 10.3897/rrpharmacology.6.54558

Research Article

Study of aversive and p38 mapk-inhibitory properties of kappa-agonist with analgesic activity – compound RU-1205

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Academic editor: Oleg Gudyrev ♦ **Received** 29 May 2020 ♦ **Accepted** 18 July 2020 ♦ **Published** 25 September 2020

Citation: Spasov AA, Zvartau EE, Grechko OIu, Eliseeva NV, Semenova YuV, Dravolina OA, Vasiliev PM, Anisimova VA (2020) Study of aversive and p38 mapk-inhibitory properties of kappa-agonist with analgesic activity – compound RU-1205. Research Results in Pharmacology 6(3): 59–65. https://doi.org/10.3897/rrpharmacology.6.54558

Abstract

Introduction: The clinical use of kappa-opioid agonists, despite their lack of significant drug potential, is limited by the development of severe sedation, dysphoria, depression, and anhedonia. To this date, there are kappa-opioid receptor agonists lacking these side effects due to the selective activation of intracellular signal transmission pathways without p38-MAPK-kinase activation.

Materials and methods: We analyzed assessment of the docking energy of compound RU-1205 to the p38-MAPK active center by the method of similarity to SB203580. The study of possible aversive properties of RU-1205 (0.01–1 mg/kg s.c.) conducted in the tests of the intravenous self-administration and drug differentiation with butorphanol (0.01–0.3 mg/kg). The study of p38 MAPK-inhibitory activity was studied by the ability of RU-1205 to change the aversive properties of U50488 (10 mg/kg i.p.) compared to MAPK-kinase inhibitor SB203580 in the conditioned place avoidance test.

Results: The spatial similarity coefficient of the RU-1205 molecule with SB203580 by the molecular conformation method was 1.14 (high similarity), and the docking energy was -8.7 Kcal/mol. RU-1205 did not possess any properties similar to those of butorphanol and did not demonstrate any primary reinforcing aversive properties in the development of intravenous self-administration reaction. Compound RU-1205 did not demonstrate any aversive properties in the conditioned place avoidance test, and reduced the development of aversion caused by U-50488, when they were used together.

Discussion: The *in silico* analysis suggested that, in addition to agonism towards the kappa-opioid receptor, RU-1205 compound exhibits the properties of a p38 MAPK kinase inhibitor, which means it may have a double pharmacological activity.

Conclusion: Kappa agonist – compound RU-1205 – is not a trigger of the development of behavioral patterns in animals corresponding to the development of addiction/dysphoria. The mechanism of such an activity may be associated with an inhibitory effect of compound RU-1205 on neuronal p38-MAPK-kinase.

Keywords

aversion, dysphoria, kappa-opioid receptor, p38 MAPK-kinase.

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Introduction

Although kappa opioid receptor (KOR) agonists are highly effective analgesics with a low risk of dependence and abuse, the advancement of the kappa opioid ligand class is largely limited to kappa-receptor-specific neuropsychiatric adverse effects, including sedation and dysphoria (Schattauer et al. 2017; Gross et al. 2019; Margolis and Karkhanis 2019). Currently, there is evidence that kappa agonists that do not activate p38 MAPK kinase (Ehrich et al. 2015), the stimulation of which is the cause of kappa-mediated aversive reactions, will not cause dysphoria, sedation and, at the same time, maintain a high enough analgesic efficacy for treating various conditions associated with severe pain (Bruchas et al. 2007; White et al. 2015; Zan et al. 2016; Abraham et al. 2018). A preliminary experimental study conducted at the Volgograd State Medical University, Department of Pharmacology and Bioinformatics, resulted in isolating a novel compound RU-1205 (fluorophenyl derivative of [1,2-*a*] imidazobenzimidazole) with a pronounced analgesic activity superior to that of butorphanol and morphine in *in vivo* experiments (Grechko et al. 2016; Spasov et al. 2018). The kappa-receptor profile of compound RU-1205 was experimentally proven *in vitro* on a model of rabbit vas deferens (EC_{50} of substance RU-1205, butorphanol and U 50.488 were 2 nM, 9800 nM and 7 nM, respectively), as well as in an *in vivo* a bunch of nociceptive tests using kappa-selective NorBNI antagonist (Grechko et al. 2017).

The aim of this study was to assess the possible effect of compound RU-1205, demonstrating a pronounced kappa-opioid agonistic activity, on the neuronal p38 MAPK-kinase, as well as the identification of pharmacological properties of the compound, considered as specific predictors of aversion.

Materials and methods

The object of the study was 9-(2-morpholinoethyl) -2-(4-fluorophenyl) imidazo [1,2-*a*] benzimidazole dihydrochloride (compound RU-1205) (Patent of the Russian Federation No. 2 413 512 C1 of July 29, 2009). Butorphanol tartrate (Federal State Unitary Enterprise Moscow Endocrine Plant, Russia) and a selective kappa agonist, compound U-50488 (SIGMA, USA), were used as the comparison preparations; a MAPK-kinase inhibitor, SB203580 (SIGMA, USA) was used in studying the change in the aversive properties of compound U-50488. The experiments were conducted on male Wistar rats (280–330 g; drug differentiation) and outbred male white mice (25–30 g; development of an intravenous self-introduction reaction and a conditioned place avoidance reaction). The animals were obtained from Rappolovo Laboratory Animal Nursery of the Russian Academy of Medical Sciences (St. Petersburg, Russia). All the experiments were carried out in accordance with the *Principles of Good Laboratory Practice* (National Standard of the Russian Federation GOST R 53434-2009) and the provisions of the international convention on *The Rules for Working with Experimental Animals* (European Communities Council Directives, November 24, 1986, 86/609/EEC).

Docking-analysis

To assess the docking energy of compound RU-1205 to the p38 MAPK active center we employed the method of similarity with the reference. An α-p38MAPK inhibitor, SB203580 (PDB, 2011), was used as a reference preparation. The calculations were performed using HyperChem 7.0 (Evaluation Copy). Docking of the substance into the α-p38MAPK catalytic domain was performed using the AutoDock Vina 1.1.1 software package, complete with additional AutoDock Tools and PyMol tools (Trott and Olson 2010) on the 24-core computing cluster with a total capacity of 190 Gflops. We used a 3D model of α-p38-MAP kinase obtained from the open Protein Data Bank in Europe (PDBe).

Conditioned place avoidance test

The method of generating a conditioned place avoidance response was carried out in a modified initially balanced selection of the compartments of the experimental chamber (two compartments are distinguished by visual and tactile cues), which made it possible to be confident in determining the selective "rewarding" or aversive effects of the compound. The conditioning stage included 8 sessions of 30 minutes with a closed partition, at 10.00 and at 16.00 after the test compound or solvent was introduced into the appropriate compartment (in the equally probable sequence) (Voronina and Guzevatykh 2012). The compounds were studied in the following doses: RU-1205 in the dose of 5 mg/kg, U-50488 in the dose of 10 mg/kg (by intraperitoneal injection), distilled water was used as a solvent. To study the systemic effect of RU-1205 on the development of kappa-mediated aversion in comparison with the p38 MAPK kinase inhibitor SB203580, compounds RU-1205 and SB203580 (0.05 mg/kg, intraperitoneally) were administered during the conditioning period from day 2 to day 5 of the experiment 20 minutes before the introduction of U-50488 (10 mg/kg, intraperitoneally). In all the tests on the last day, the time spent by the animals in each of the compartments with an open partition for 900 seconds was recorded. A statistically significant avoidance by the animals of the compartment associated with the substance was taken as a criterion for the development of aversion.

Intravenous self-administration

The effects of compound RU-1205 in the dose range of 0.01–1 mg/kg with subcutaneous administration were evaluated. The work was carried out in a facility (Kuz'min and Zvartau 1991) for pairwise testing of animals, the facility being equipped with a photosensor fixing nose-pokes of the mice and controling the operation of a two-syringe microinjector (1.6 μl/injection) in response to

each nose-poking of one of the mice in the experimental pair. The procedure consisted of an initial testing (10-min pretest), and a testing, followed by intravenous infusions of a solution of the test substance (30-min test). During the test, one of the mice in the pair received intravenous infusions in response to its own nose-poking ("active" – AM), the other – according to the rhythm of AM nose-poking ("passive" – PM), without any connection to its own nose-pokes. The numbers of nose-pokes of AM and PM were recorded, on the basis of which the self-administration criterion (R-criterion) was calculated by the formula:

$$R = \lg\left(\frac{A_2}{P_2}\right) - \lg\left(\frac{A_1}{P_1}\right),$$

where A_1 and A_2 are the numbers of nose-pokes for AM in the pretest and the test with intravenous infusions of the solution of the test substance respectively, and P_1 and P_2 are the same indicators for PM. The action of the studied compound was regarded as positively reinforcing in the case when the R value was significantly greater than that when self-administering a 0.9% solution of NaCl (values approximately equal to zero) and the cumulative dose of the substance (mg/kg) consumed by AM during the self-administration session.

Drug differentiation

The effects from compound RU-1205 were evaluated in the dose range of 0.01–1 mg/kg with subcutaneous administration compared with butorphanol. The work was carried out in Skinner's automated soundproof operant chambers (RITEK, Russia). The animals were trained to press the pedals to obtain food pellets in the mode of gradually increasing the value of the fixed ratio (FR) from 1 to 10 (every 10th pressing was reinforced), which were subsequently associated either with injections of an isotonic NaCl solution or with the introduction of butorphanol. Sessions with solvent (F) and butorphanol (H) were carried out in an alternating sequence, divided into blocks lasting for 2 months: (1) FNFNFFN, NFFFFNFF, NNNFNFN, FNNFFFF; (2) NFNFNNF, FNNNFNN, FFFNFNF, NFFNNFN. Thirty minutes after the injection, the animals were placed in the operant chambers for 15 min. During the sessions, the animal had free access to both pedals; however, only pressing one of them (depending on the injection received) was supported by the provision of food pellets in the reinforcement mode of FR 10. The order of the tests was determined according to the "Latin square" scheme.

Statistical processing

Processing of the obtained data was performed using the SPSS software package (version 16, USA). The statistical significance of the action of the substances and its dose dependence were evaluated using analysis of variance, taking into account the priority of administration. The intergroup comparisons (Dunnett test) were carried out only under the condition that a significant effect was revealed by the analysis of variance.

Results

Docking analysis

A comprehensive *in silico* assessment revealed a high level of spatial similarity of RU-1205 compound with the three-dimensional structures of the p38MAPK kinase inhibitor – SB203580. Formation of a stable complex of RU-1205 compound with the catalytic domain of the enzyme occurred with a binding energy of -8.7 Kcal/mol, which was comparable with the calculated value of the interaction energy of SB203580 with the p38 MAPK kinase binding site (Table 1).

Table 1. Estimated Affinity of Neuronal p38 Inhibitory p38 MAPK Kinase Activity of Compounds RU-1205 and SB203580.

Substance code	Interaction with p38 MAPK kinase	
	Similarity coefficient	Docking Energy, Kcal/mol
RU-1205	1.14	-8.8
SB203580	–	-9.0

Note: for calculating the total coefficient of structural similarity of RU-1205 compound to the reference drug, we used of 2D similarity (Tanimoto coefficient) and 3D similarity coefficients (rms distance between the matching atoms, by surface area and by volume of matching fragments of molecules); all the indicators were normalized to the median.

Technique of the conditioned place avoidance response

Before conditioning, the mice of all groups spent equal time in each compartments of the experimental chamber (Fig. 1).

After the test session, it was found that RU-1205 compound did not cause a statistically significant preference/avoidance of any of the chamber compartments, while

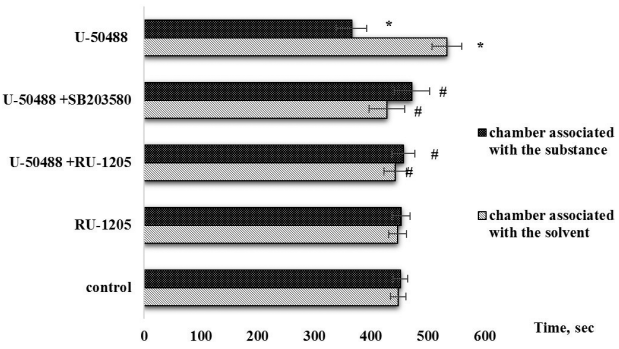

Figure 1. The effect of the compound RU-1205 and U-50488 on the time spent by animals in the compartments of the facility to study the avoidance response. **Note:** * – statistically significant compared with control, # – statistically significant compared with U-50488, U Mann-Whitney test ($p \leq 0.05$).

animals treated with the selective kappa agonist U-50488 significantly spent 22% less time in the chamber associated with the substance, which confirms its aversive properties (Abraham et al. 2018). In this case, the RU-1205 compound increased the time spent in the chamber associated with the introduction of U-50488, similarly to the action of the p38 MAPK kinase inhibitor SB203580, which statistically significantly offset the development of kappa-mediated aversion caused by U-50488.

Development of the intravenous RU-1205 self-administration reaction

The total results are presented in Table 2. RU-1205 did not cause the development of self-administration significantly different from the self-introduction of the solvent, and, therefore, did not have a significant reinforcing effect in the range of the studied concentrations (R-criterion – $F(6.58) = 0.618$, $P = 0.715$).

Table 2. Effects of RU-1205: Initiation of the Intravenous Self-administration Reaction in Mice.

Substance (mg/ml)	Concentration (mg/ml)	R-criterion	Cumulative Dose (mg/kg)
RU-1205	0 (water)	-0.02 ± 0.06	–
	0 (isot. solution NaCl)	-0.03 ± 0.05	–
	0.01	-0.12 ± 0.08	0.02 ± 0.00
	0.03	-0.04 ± 0.09	0.10 ± 0.02
	0.1	0.00 ± 0.08	0.33 ± 0.06
	0.3	-0.09 ± 0.06	0.83 ± 0.16
	0.56	-0.02 ± 0.09	1.77 ± 0.28
	1	0.08 ± 0.09	4.31 ± 0.52

Note: The data are presented as average values (M ± m) of the R-criterion and cumulative dose of the substance consumed by the active mouse per session. N = 6–18.

Assessing the similarity of the differentiating interoceptive stimulus properties of RU-1205 with those of butorphanol

The rats were trained in the procedure of drug differentiation within 54 days on average (28–75 days). During control sessions with a training dose of butorphanol (0.3 mg/kg) or with a 0.9% solution of NaCl, the number of steps on the corresponding reinforced pedal exceeded 95% of the total number of steps on both pedals per session. During the tests, an increase in the dose of butorphanol (0.01–0.3 mg/kg) dose-dependently increased the probability of rats choosing the pedal associated with butorphanol (Fig. 2).

RU-1205 did not possess differentiating interoceptive stimulus properties similar to those of butorphanol (Fig. 3). After the introduction of RU-1205 (0.01–10 mg/kg), none of the animals selected the pedal associated with butorphanol at the beginning of the test; in general, during the experimental session, the percentage of pedal selection associated with the introduction of butorphanol did not differ from that after the introduction of the solvent ($F(5.52) = 0.923$, $P = 0.474$). Within the range of doses studied, RU-1205 did not affect the frequency of the operant reaction ($F(5.52) = 1.114$, $P = 0.364$).

Discussion

Although KOR agonists have long been recognized as analgetics with low potential for abuse, their use may be associated with the development of serious psychotropic side effects, including dysphoria, anhedonia, and hallucinations. In recent years, advances in immunohistochemistry and neuropharmacology have made it possible to determine the putative neurobiological mechanism of the formation of kappa-induced dysphoria and aversion, which means the activation of the mitogen-activated protein kinase p38MAPK in the ventral tegmental region (Schattauer et al. 2017). According to the literature, selective inhibitor of p38 MAPK kinase – SB203580 – significantly inhibited p38 MAPK kinase, which showed in reduced dysphoria manifestations when used together with highly selective kappa agonists, while it does not affect the severity of analgesia (Margolis and Karkhanis 2019). It is assumed that the elimination of p38MAPK activity does not affect the formation of analgesia (Zan et al. 2016). To date, there are agonists of kappa-opioid receptors that have a pronounced

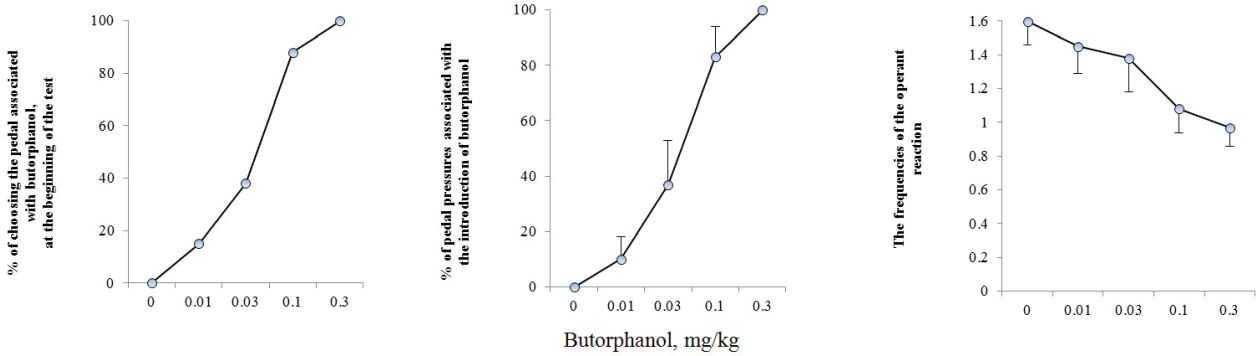

Figure 2. Differentiating stimulus properties of butorphanol. **Note:** butorphanol (0.01–0.3 mg/kg) or its solvent were administered subcutaneously 30 minutes before the test. Data are presented as: % of animals that performed the first 10 consecutive pedal pressures associated with the administration of a training dose of butorphanol (0.3 mg/kg; left); % of pedal pressures associated with the introduction of butorphanol (M ± m; center); the frequencies of the operant reaction (pressing/sec, M ± m; right). N = 8.

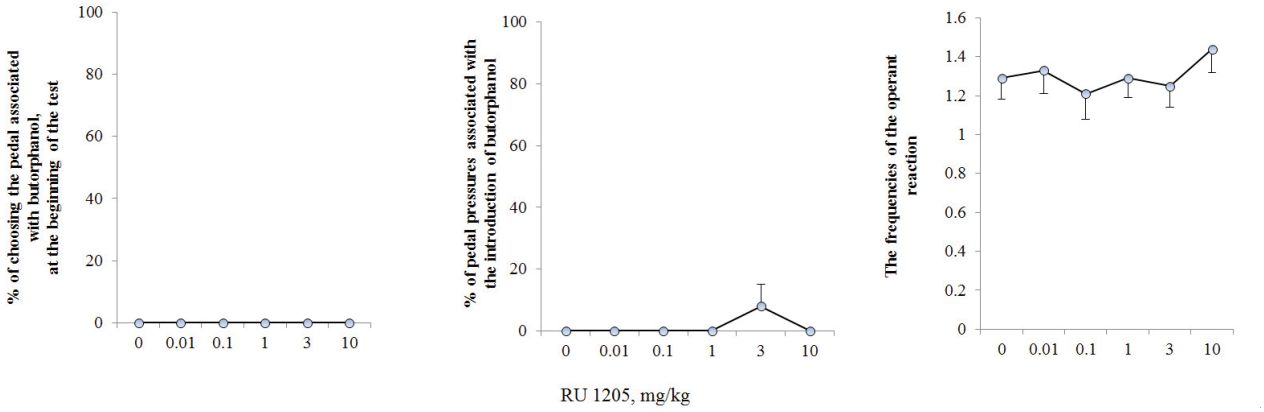

Figure 3. Identification of the similarity of differentiating stimulus properties of the test substance RU-1205 and butorphanol. **Note:** Animals preliminarily trained to distinguish between the interceptive effects of butorphanol were injected with the test substance RU-1205 (0.01–10 mg/kg) or its solvent, and the probability of a behavioral reaction typical for the introduction of a training dose of butorphanol (0.3 mg/kg) was assessed. Data are presented as: % of animals that performed the first 10 consecutive steps on the pedal associated with the introduction of butorphanol (left); % of pedal pressures associated with the introduction of butorphanol (M ± m; center); the frequencies of the operant reaction (pressing/sec, M ± m; right). N = 8–12.

analgesic effect without causing dysphoria (Ehrich et al. 2015; Gross et al. 2019), which either have a multi-targeted effect with the properties of selective kappa agonists and p38 MAPK kinase inhibitors, or are "functionally selective" kappa receptor ligands. The term "functional selectivity" or "biased agonism" describes the ability of a ligand of a G-protein-coupled receptor to selectively activate a subset of signaling cascades in a particular GPCR, as opposed to activating all downstream signaling cascades (eg. G-proteins, arrestin and/or kinase) (Bruchas et al. 2007). It has been suggested that p38 MAPK activation is mediated by β-arrestin-2 signaling, which allows us to consider the therapeutic potential of KOR ligands functionally selective towards G protein and away from β-arrestin2 pathways as analgetics without dysphoric side effects (Gross et al. 2019).

The fact that selective p38 MAPK inhibitors belong to fluorophenyl derivatives of imidazole systems (Abraham et al. 2018) served as a prerequisite for the inclusion of these fragments in the molecular composition with the aim of creating structurally new kappa receptor agonists with the properties of p38 MAPK inhibitors. The *in silico* analysis suggested that, in addition to agonism with respect to the kappa-opioid receptor, RU-1205 compound exhibits the properties of a p38 MAPK kinase inhibitor, which means it may have a double pharmacological activity. In the docking analysis, it was found that the interaction energy of RU-1205 substance with the catalytic domain of p38-MAPK kinase was high and corresponded to the reference drug SB203580.

The pharmacological activation of KOR in humans causes dysphoria and anxiety similar to those found in experimental models. Addictive and aversive effects can be fixed by assessing the ability of the substances to act as primary and secondary "positive" or "negative" reinforcers. At the same time, the same preclinical tests ("intravenous self-administration", "drug differentiation" for the primary, and "conditioned place avoidance/preference" for secondary reinforcements) are used to assess the ability to form both of these conditions. A kappa agonist and mu-opiate receptor antagonist butorphanol was used as a comparison substance in connection with the generality of intermediary receptors and the field of therapeutic use. In the works by Ahsan H. M. (Ahsan HM et al. 2014), it was experimentally confirmed that the results of the "intravenous self-administration" reaction correlated with the results of conditioned place avoidance test. Negative reinforcement is associated with the manifestation of dysphoric activity. It was established that compound RU-1205 in all the conducted behavioral tests ("conditioned place avoidance", "intravenous self-administration" and "drug differentiation") showed no aversive activity. The test substance did not cause primary reinforcement in the""intravenous self-administration" during the current study.

Conclusion

The totality of the results suggests that the effect on kappa receptors and p38 MAPK is promising for the development of highly effective opioid analgetics without narcogenic potential and kappa-mediated aversive disorders. Based on the experiments performed, we can conclude that this substance in the studied dose range for these routes of administration does not have pharmacological properties, which can be regarded as specific predictors of aversion. The mechanism of such an altered pharmacological profile of RU-1205 is possibly associated with the effect on p38-MAPK kinase. It includes not only the absence of an aversive effect, but also the absence of a rewarding effect, as well as a set of interoceptive stimulus properties different from those of butorphanol.

Conflict of interests

The authors declare no conflict of interests.

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