KJP

Korean J Pain 2024;37(1):26-33
https://doi.org/10.3344/kjp.23262
pISSN 2005-9159, eISSN 2093-0569

Experimental Research Article

Anti-inflammatory and antinociceptive effects of sitagliptin in animal models and possible mechanisms involved in the antinociceptive activity

Valiollah Hajhashemi[1], Hossein Sadeghi[2], and Fatemeh Karimi Madab[1]

[1]Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Pharmacology, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran

ABSTRACT

Background: Sitagliptin is an antidiabetic drug that inhibits dipeptidyl peptidase-4 enzyme. This study aimed to investigate the antinociceptive and anti-inflammatory effects of sitagliptin in formalin and carrageenan tests and determine the possible mechanism(s) of its antinociceptive activity.
Methods: Male Swiss mice (25–30 g) and male Wistar rats (180–220 g) were used for formalin and carrageenan tests, respectively. In the formalin test, paw licking time and in the carrageenan test, paw thickness were considered as indexes of pain behavior and inflammation respectively. Three doses of sitagliptin (2.5, 5, and 10 mg/kg) were used in these tests. Also, several antagonists and enzyme inhibitors were used to evaluate the role of adrenergic, serotonergic, dopaminergic, and opioid receptors as well as the NO/cGMP/K_{ATP} pathway in the antinociceptive effect of sitagliptin (5 mg/kg).
Results: Sitagliptin showed significant antinociceptive and anti-inflammatory effects in the formalin and carrageenan tests respectively. In the carrageenan test, all three doses of sitagliptin significantly ($P < 0.001$) reduced paw thickness. Pretreatment with yohimbine, prazosin, propranolol, naloxone, and cyproheptadine could not reverse the antinociceptive effect of sitagliptin (5 mg/Kg), which indicates that adrenergic, opioid, and serotonin receptors ($5HT_2$) are not involved in the antinociceptive effects. L-NAME, methylene blue, glibenclamide, ondansetron, and sulpiride were able to reverse this effect.
Conclusions: NO/cGMP/K_{ATP}, $5HT_3$ and D_2 pathways play an important role in the antinociceptive effect of sitagliptin. Additionally significant anti-inflammatory effects observed in the carrageenan test might contribute in reduction of pain response in the second phase of the formalin test.

Keywords: Analgesics; Anti-Inflammatory Agents; Carrageenan; Dipeptidyl-Peptidases and Tripeptidyl-Peptidases; Formaldehyde; Mice; Pain; Pain Measurement; Sitagliptin Phosphate.

Received September 11, 2023; Revised November 24, 2023; Accepted December 3, 2023

Handling Editor: Jong Yeon Park

Correspondence: Valiollah Hajhashemi
Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Hezar-Jarib Ave., Isfahan 81746-73461, Iran
Tel: +98-313-7927080, Fax: +98-313-6680011, E-mail: vhajhashemi@gmail.com

INTRODUCTION

Sitagliptin is a well-known dipeptidyl peptidase-4 (DPP-4) inhibitor that is widely used in diabetic patients as monotherapy or in combination with other antidiabetic medications like metformin. Despite an increased risk of infections like nasopharyngitis and urinary tract infections, the drug has an acceptable safety profile and has been well tolerated [1]. Previous researches have documented that DPP-4 inhibitors have antioxidant, antiapoptotic, and antifibrotic activities [2,3]. Additionally, antinociceptive and anti-inflammatory effects have been reported for sitagliptin [4,5].

Multiple neurotransmitters and receptors have been implicated in the signaling of pain, according to earlier studies [6,7]. In the pain pathway, following stimulation of nociceptors, the message transmits to the dorsal horn and reaches the thalamus *via* the spinothalamic pathway. Additionally, ascending information reaches the neurons of the rostral ventral medulla and the periaqueductal gray in the midbrain that evokes the descending pathway which eventually modulates the output from the spinal cord. It has been documented that the endogenous opioids, cannabinoids, 5-hydroxytryptamine (5-HT), and norepinephrine have a crucial role in pain signaling pathways in brain sites. Also, in the dorsal horn, tachykinins (substance P and other neuropeptides), calcitonin gene-related peptide, glutamate, and 5-HT modulate transmission of pain signals through spinothalamic path [6]. Opioids, as potent analgesics, activate receptors located at peripheral, spinal, and supraspinal sites to suppress pain [8]. Also, serotonin controls pain through 5-HT_{2A} and 5-HT_3 receptors. Thermal and chemical pain may be reduced by antagonists of these receptor. $5HT_3$ antagonists modulate pain and inflammation through substance P, which is especially effective in chronic pain. Low dosages of these antagonists may release more endogenous opioids in the spinal cord, while greater doses may alleviate pain by activating neuronal activity in nociceptive pathways [9]. In addition, the adrenergic system has an impact on the nociceptive system [10].

Another neurotransmitter that is involved in the mediation of nociceptive behavior is dopamine. Studies on both animals and humans have shown that the striatal D_2 and D_3 receptors play an essential regulating function in the regulation of pain [11].

One of the most well-studied signaling molecules that contributes in pain signaling is nitric oxide (NO) and it has been linked to analgesic effects in several studies. Analgesia is achieved when the intracellular concentration of cyclic guanosine monophosphate (cGMP) is raised, which is the result of activation of the enzyme guanylate cyclase by NO. The analgesic action of opioid receptor agonists has been demonstrated to be mediated in part through the NO and cGMP pathways. NO-releasing factors have also been shown to alleviate pain [12,13].

While previous studies have looked into sitagliptin's antinociceptive and anti-inflammatory effects, the effects have not been evaluated in the carrageenan and formalin models, which are the most reliable and widely used models of inflammation and pain, and detailed information about the mechanism(s) of the antinociceptive effects is not available.

According to the above considerations, the aims of the present study were: (1) to evaluate the antinociceptive effect of sitagliptin in the formalin test in mice, (2) to investigate its anti-inflammatory activity in the carrageenan test in rats, and (3) to examine some possible mechanisms involved in the antinociceptive activity produced by sitagliptin in the formalin model of nociception.

MATERIALS AND METHODS

1. Drugs

Sitagliptin was from Arya Pharmaceutical Company. Ondansetron (Tehran Chemie Pharmaceutical Co.), propranolol and naloxone (Tolid Daru) were also used. Raha and Farabi Pharmaceutical companies provided cyproheptadine and tadalafil respectively. The remaining chemicals including L-NAME, arginine, methylene blue, glibenclamide, sulpiride, prazosin, and yohimbine were purchased from Sigma Chemical Co.

2. Animals

In the animal house of the School of Pharmacy in Isfahan, Iran, 30 male Wistar rats (200 ± 20 g) and 168 male Swiss mice (25–30 g, 10–12 weeks old) were maintained under controlled environmental conditions at 23°C–25°C and a 12 h light/dark cycle. They had free access to both standard rodent chow pellets and drinking water. All animal studies were conducted in accordance with The National Ethical Committee of Iran (Ethics code: IR.MUI.RESEARCH.REC.1400.555) recommendations for the care and use of laboratory animals. Every attempt was made to minimize the pain suffering of the animals and decrease the total number of animals employed.

3. Experimental design

At first, antinociceptive effect of three doses of sitagliptin (2.5, 5, and 10 mg/kg) was evaluated in the formalin test. Based on the results, a dose of 5 mg/kg of sitagliptin was selected for mechanistic experiments.

In the mechanistic section, different groups of mice (n = 6) were pretreated with prazosin (2 mg/kg), yohimbine (5 mg/kg), propranolol (2 mg/kg), naloxone (5 mg/kg), sulpiride (20 mg/kg), cyproheptadine and ondansetron (2 mg/kg), arginine (100 mg/kg), L-NAME (20 mg/kg), methylene blue (5 mg/kg), tadalafil (2 mg/kg), or glibenclamide (10 mg/kg) thirty minutes prior to sitagliptin administration and then the formalin test was performed. All drugs were injected intraperitoneal except formalin that was injected subcutaneously (s.c.). The doses were selected based on previous studies [14,15]. In another series of animals, the same doses of the above-mentioned drugs were used without sitagliptin.

4. Formalin test

Formalin (2.5% v/v, 20 μL) was administered s.c. into the right hind paw of mice 30 minutes after the injection of sitagliptin. The time spent for paw licking was measured at 0–5 and 20–40 minutes after formalin injection and considered the acute and chronic phases respectively [16,17].

5. Carrageenan-induced paw edema

In the carrageenan test, rats were divided into 5 groups of 6, including three groups receiving sitagliptin (2.5, 5, and 10 mg/kg), a negative control group (receiving only carrageenan) and a positive control group (indomethacin group, 10 mg/kg). Thirty minutes after intraperitoneal administration of the drugs, 100 μL of carrageenan suspension (1% w/v) was injected into the sub-plantar space of the right paw of animals, and paw thickness was measured once before carrageenan administration and then at time intervals of 1 and 4 hours after administration. Edema was calculated and compared with the control group [18,19].

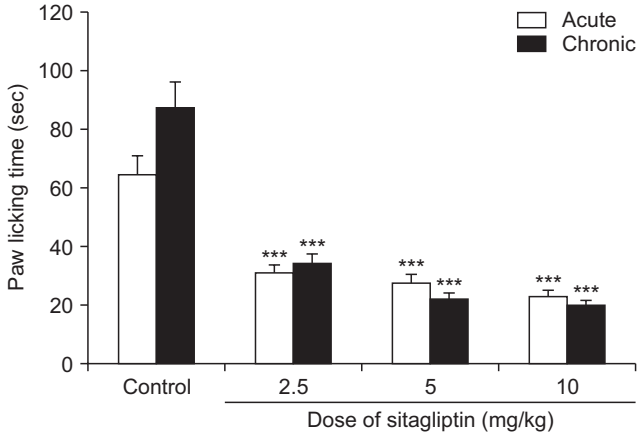

Fig. 1. The antinociceptive effect of three different doses of sitagliptin in formalin test. Data show mean ± standard error of the mean of 6 mice per group. ***$P < 0.001$ compared to control group (one way analysis of variance and Scheffe *post hoc*).

6. Statistical analysis

Results are expressed as mean ± standard error of the mean. The data was analyzed using one-way analysis of variance and the Scheffe *post hoc* test. *P* values less than 0.05 were judged to be statistically significant. The statistical analysis was performed using SPSS 25.0 software (IBM Corp.). A software package (Excel 2020; Microsoft) was used for graphing.

RESULTS

1. Antinociceptive effect of sitagliptin and the effect of antagonists and enzyme inhibitors on it in the formalin test

The antinociceptive effect of sitagliptin was statistically significant for all applied doses and in both phases of formalin test ($P < 0.001$) (**Fig. 1**).

To evaluate the role of adrenoceptors, 5 mg/kg of yohimbine (selective alpha 2 antagonist), 2 mg/kg of prazosin (selective alpha 1 antagonist), and 2 mg/kg of propranolol (non-selective beta-adrenergic receptor antagonist) were administered.

None of the mentioned drugs could reverse the antinociceptive effect of sitagliptin (**Fig. 2**). The paw licking time was not significantly different between control and groups that received antagonists alone.

Cyproheptadine, a non-selective antagonist, and ondansetron as a $5HT_3$ antagonist, were injected at a dose of 2 mg/kg to assess the contribution of serotonin receptors.

Cyproheptadine in both phases and ondansetron in the acute phase could not reverse the antinociceptive effect of sitagliptin, but ondansetron reversed this effect in the chronic phase of the formalin test ($P = 0.006$) (**Fig. 3**).

The contribution of opioid receptors in the antinociceptive effect of sitagliptin was assessed by naloxone (5

 https://doi.org/10.3344/kjp.23262

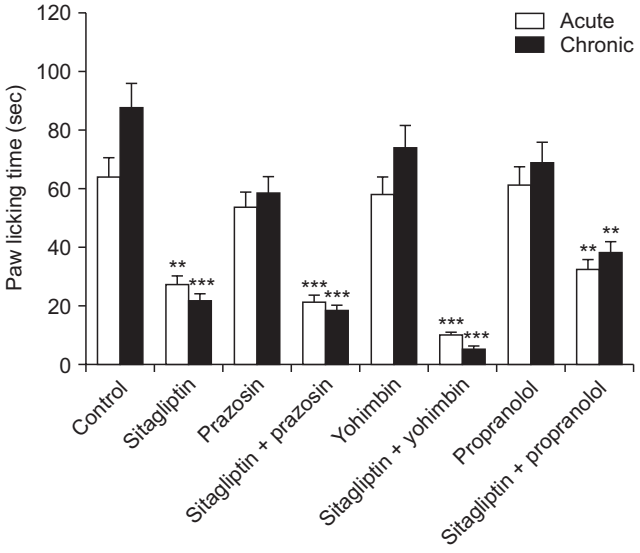

Fig. 2. Evaluation of the effect of adrenergic system on the antinociceptive effect of sitagliptin. Data show mean ± standard error of the mean of 6 mice per group. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to control group (one way analysis of variance and Scheffe *post hoc*).

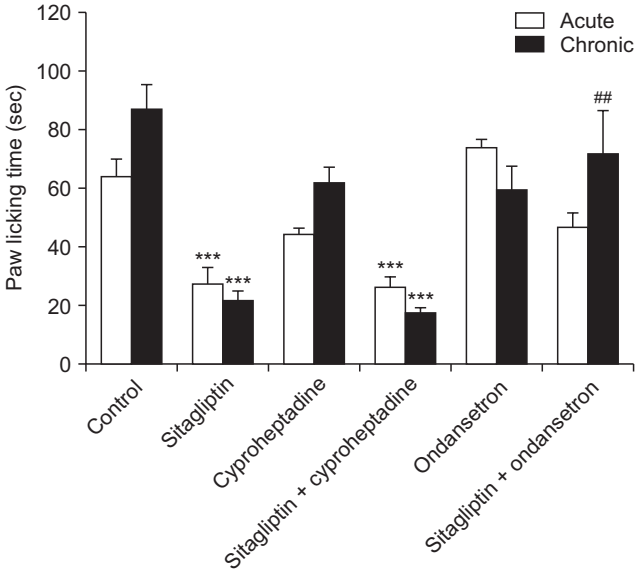

Fig. 3. The effect of cyproheptadine or ondansetron on the antinociceptive effect of sitagliptin. Data show mean ± standard error of the mean of 6 mice per group. $^{***}P < 0.001$ compared to control group. $^{\#\#}P < 0.01$ compared to sitagliptin group (one way analysis of variance and Scheffe *post hoc*).

mg/kg). Naloxone alone could not produce any significant change in formalin-induced pain behavior. Also, pretreatment with naloxone did not prevent the antinociceptive effect of sitagliptin in both phases of the formalin

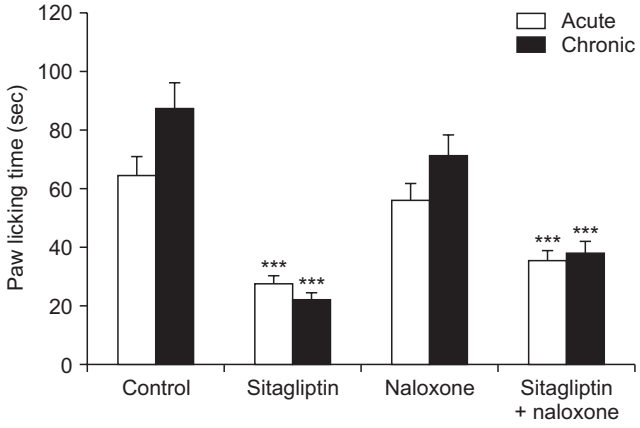

Fig. 4. The effect of naloxone on the antinociceptive effect of sitagliptin. Data show mean ± standard error of the mean of paw licking time of 6 mice per group. $^{***}P < 0.001$ compared to control group (one way analysis of variance and Scheffe *post hoc*).

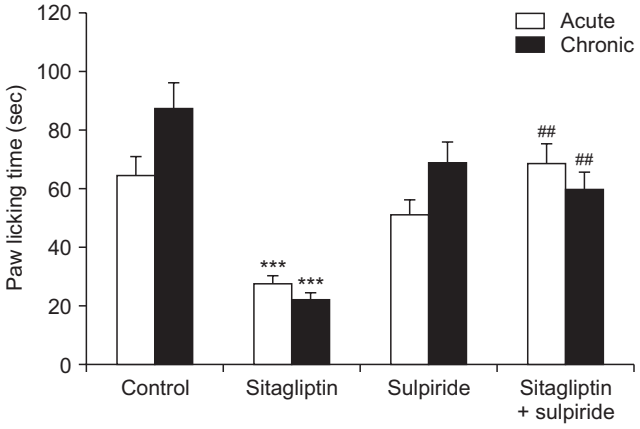

Fig. 5. The effect of sulpiride on the antinociceptive effect of sitagliptin. Data show mean ± standard error of the mean of paw licking time of 6 mice per group. $^{***}P < 0.001$ compared to control group. $^{\#\#}P < 0.01$ compared to sitagliptin group (one way analysis of variance and Scheffe *post hoc*).

test (**Fig. 4**).

Sulpiride (20 mg/kg) was used as a selective D_2 antagonist to assess the involvement of dopamine receptors. As it is seen in **Fig. 5**, this drug antagonized the antinociceptive effect of sitagliptin in both phases of the formalin test ($P = 0.002$ in the acute phase and $P = 0.005$ in the chronic phase).

NO precursor arginine (100 mg/kg), NO synthase inhibitor L-NAME (20 mg/kg), guanylyl cyclase inhibitor methylene blue (5 mg/kg), and PDE5 inhibitor tadalafil (2 mg/kg) were employed in this study. To test the function of ATP-dependent potassium channels, glibenclamide (10

mg/kg) was also administered.

There is a significant difference between L-NAME-, methylene blue- and glibenclamide-pretreated groups with sitagliptin group ($P < 0.001$, $P = 0.041$, and $P = 0.003$, respectively) in the chronic phase of formalin test. Also, there was no significant difference between the duration of paw licking in the tadalafil- and arginine-pretreated groups with the sitagliptin group in both acute and chronic phases (**Fig. 6**).

2. Carrageenan-induced paw edema

The anti-inflammatory effect of sitagliptin and indomethacin (the standard drug) was investigated in the carrageenan test. Four hours after carrageenan injection, all three doses of sitagliptin, as well as indomethacin, showed significant differences in paw thickness compared to the control (**Fig. 7**).

DISCUSSION

Our results showed that sitagliptin inhibits the perception of pain in the formalin test. Also, it showed an anti-inflammatory effect in the carrageenan test as indicated by reducing paw thickness in the rats. Another finding was the role of the NO/cGMP/K_{ATP} signaling pathway, dopamine D_2, and 5-HT_3 receptors as possible antinociceptive mechanisms.

Previously anti-inflammatory and analgesic activities of sitagliptin was reported in a neutrophil accumulation model and mechanical touch sensitivity in complete Freund's adjuvant-induced arthritis [4]. In the present study two other models (the formalin and carrageenan models) were used for the first time to assess the antinociceptive and anti-inflammatory effects and the authors' findings confirmed the previous results [4].

The formalin test is a valid and sensitive measure of pain that has widespread use in pharmaceutical research. There are two distinct stages of pain behavior in this test: the acute phase, which occurs during the first five minutes after formalin injection, and the chronic phase, which occurs 20–40 minutes later. When formalin is injected into a mouse's hind paw, it activates the pain C fibers and causes the mouse to experience pain. In this test, the severity of the pain is measured by the time spent paw licking [16,20].

The well-known opioid antagonist naloxone was unable to counteract the antinociceptive effect of sitagliptin, indicating that opioid receptors are not involved in the antinociceptive effect observed with sitagliptin. The authors also administered alpha-1, alpha-2, and non-selective beta receptor antagonists (prazosin, yohimbine, and propranolol) to test the adrenergic pathway. Pretreatment with these drugs had no impact on sitagliptin's antinociceptive action, therefore the present study did not imply

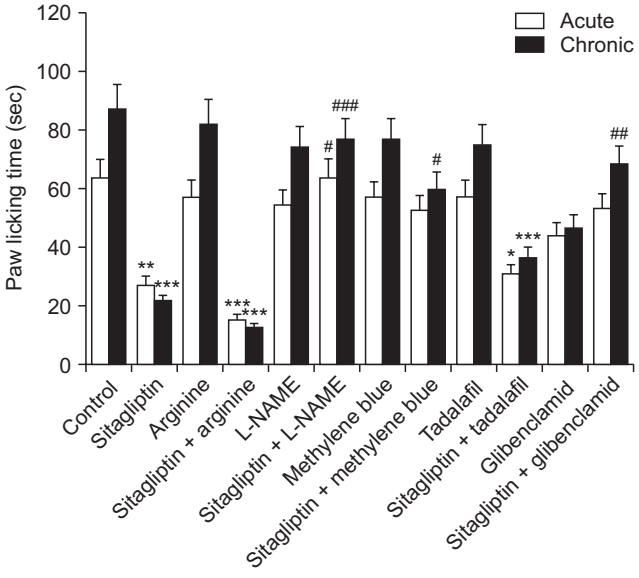

Fig. 6. The effect of the drugs acting on NO/cGMP/K_{ATP} pathway on the antinociceptive effect of sitagliptin. All data are expressed as mean ± standard error of the mean of 6 mice per group. NO: nitric oxide, cGMP: cyclic guanosine monophosphate. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to control group. #$P < 0.05$, ##$P < 0.01$ and ###$P < 0.001$ compared to sitagliptin group (one way analysis of variance and Scheffe *post hoc*).

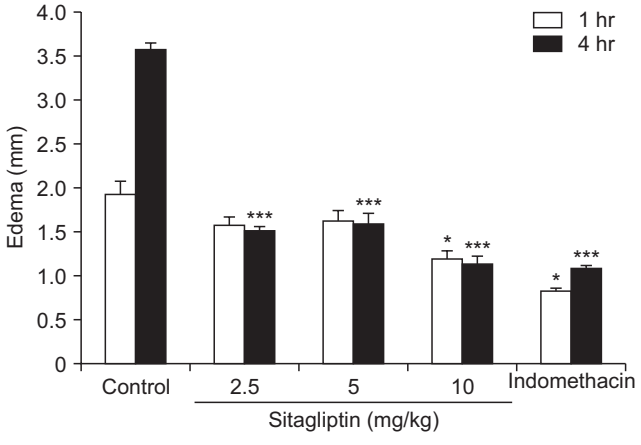

Fig. 7. Anti-inflammatory effect of three different doses of sitagliptin in carrageenan test. *$P < 0.05$ and ***$P < 0.001$ refer to statistical significance from the control (one way analysis of variance and Scheffe *post hoc*).

 https://doi.org/10.3344/kjp.23262

a role for these receptors.

The involvement of serotonin receptors in pain regulation has previously been investigated. It has been shown that stimulation of the periaqueductal grey area causes the release of serotonin (5-HT) in the dorsal horn of the spinal cord, and then activation of the 5-HT_2 and 5-HT_3 receptors blocks the spinothalamic pain pathway [21].

In this investigation, cyproheptadine and ondansetron were employed to assess the function of the above serotonergic receptors in sitagliptin's antinociceptive action. Although cyproheptadine is not a selective serotonin receptor antagonist, it has a strong antagonistic impact on various subtypes of $5HT_2$ receptors, including $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ [22]. In this study, cyproheptadine per se did not show an analgesic effect and, in contrast to these results, Tan et al. [23] reported an analgesic effect for cyproheptadine in the writhing test and hot plate test. This difference may be due to different animal models as well as different doses. The authors used a dose of 2 mg/kg of cyproheptadine in the formalin test which was less than the ED_{50} (4.4 mg/kg) reported in their writhing test.

Ondansetron inhibits $5HT_3$ receptors specifically. The authors' findings showed partial reversal of the sitagliptin antinociceptive effect by ondansetron and suggested the participation of $5HT_3$ receptors in its effect. Consistent with the present study, ondansetron has decreased the analgesic efficacy of tramadol [24].

The role of D_2 receptors in pain modulation is well established [25,26]. Morgan and Franklin [27] reported that SKF 38393 (a selective D_1 agonist) had no antinociceptive effect in a formalin test while quinpirole as a selective D2 agonist dose-dependently reduced formalin-induced pain behavior. They also documented that the pimozide (0.5 mg/kg) as a D_2 antagonist attenuated morphine and amphetamine. In the present study sulpiride was used as another selective D_2 dopamine receptor blocker and significantly prevented sitagliptin-induced antinociception in both phases of the formalin test. Therefore, it is speculated that D_2 receptors might mediate sitagliptin's pain suppressing effect.

In recent years, many investigators have focused on the central and peripheral role of the NO/cGMP/K_{ATP} channel pathway in pain modulation and it appears that NO, depending on its tissue concentration and the animal model of pain, has a dual effect on pain perception. Therefore, both pro-nociceptive and antinociceptive effects have been reported for arginine as the precursor of NO biosynthesis. Also, L-NAME as a well-known inhibitor of NO synthase has produced both analgesic and anti-analgesic activities [28,29].

Our findings clearly demonstrated that L-NAME reversed the antinociceptive effects of sitagliptin. Consistent with these results, other studies have shown that L-NAME inhibited the pain-suppressing effects of ketorolac, diclofenac, and ketamine [30–32]. In the present study, L-NAME did not produce anti-nociception by itself, and this finding is inconsistent with previous reports [33,34] and might be explained by differences in the route of drug administration or drug dose.

The above effect was confirmed by reversal of sitagliptin antinociception by methylene blue (a guanylyl cyclase inhibitor) and glibenclamide (a K_{ATP} channels inhibitor).

Previous studies have shown that activation of the NO/cGMP pathway results in an opening of potassium channels and hyperpolarization of neuron membranes. Alves et al. [35] reported that diazoxide as a potassium channel opener showed antinociceptive effect. Also, Yamazumi et al. [36] reported that K_{ATP} channels contribute to the spinal antinociceptive effect of fentanyl, bethanechol, and clonidine, which means that these channels have an impact on both opioid and non-opioid analgesics. Additionally, these channels are involved in the antinociceptive effects of curcumin [37].

The present study used the carrageenan test to evaluate the anti-inflammatory effect of sitagliptin. The carrageenan test is a reliable and valid test to investigate inflammation in animals. Carrageenan causes acute and local inflammation [19]. All three doses of sitagliptin (2.5, 5, and 10 mg/kg) suppressed carrageenan-induced edema and the present study's results are consistent with the study of Makdissi et al. [38] in terms of the anti-inflammatory effect of this drug. Since the second phase of the formalin test is of inflammatory origin, it seems that anti-inflammatory the effect of sitagliptin might also contribute to the antinociceptive effect observed in the second phase of formalin test.

In conclusion, activation of dopamine D_2 and serotonin $5HT_3$ receptors as well as the NO/cGMP pathway are involved in the antinociceptive activity of sitagliptin. Also, the anti-inflammatory effect observed in the carrageenan test indicates that this effect has an important role in controlling inflammatory pain.

DATA AVAILABILITY

Data files are available from Harvard Dataverse: https://doi.org/10.7910/DVN/HLHKFL.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

FUNDING

This research was financially supported by Isfahan University of Medical Sciences, Isfahan, Iran (Grant no: 3400919).

AUTHOR CONTRIBUTIONS

Valiollah Hajhashemi: Methodology; Hossein Sadeghi: Writing/manuscript preparation; Fatemeh Karimi Madab: Investigation.

ORCID

Valiollah Hajhashemi, https://orcid.org/0000-0001-5783-2324
Hossein Sadeghi, https://orcid.org/0000-0001-8465-5933
Fatemeh Karimi Madab, https://orcid.org/0009-0002-2695-7807

REFERENCES

1. Scott LJ. Sitagliptin: a review in type 2 diabetes. Drugs 2017; 77: 209-24.
2. El-Agamy DS, Abo-Haded HM, Elkablawy MA. Cardioprotective effects of sitagliptin against doxorubicin-induced cardiotoxicity in rats. Exp Biol Med (Maywood) 2016; 241: 1577-87.
3. Kelleni MT, Amin EF, Abdelrahman AM. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in rats: impact of oxidative stress, inflammation, and apoptosis. J Toxicol 2015; 2015: 424813.
4. Újhelyi J, Újhelyi Z, Szalai A, László JF, Cayasso M, Vecsernyés M, et al. Analgesic and anti-inflammatory effectiveness of sitagliptin and vildagliptin in mice. Regul Pept 2014; 194-195: 23-9.
5. Kawasaki T, Chen W, Htwe YM, Tatsumi K, Dudek SM. DPP4 inhibition by sitagliptin attenuates LPS-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 2018; 315: L834-45.
6. Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. Int J Mol Sci 2018; 19: 2164.
7. Riedel W, Neeck G. Nociception, pain, and antinociception: current concepts. Z Rheumatol 2001; 60: 404-15.
8. Beck TC, Dix TA, Reichel CM. Targeting peripheral kappa opioid receptors for the treatment of chronic pain: review article. Adv Nanomed Nanotechnol Res 2019; 1: 16-9.
9. Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. Behav Pharmacol 2011; 22: 390-404.
10. Pertovaara A. The noradrenergic pain regulation system: a potential target for pain therapy. Eur J Pharmacol 2013; 716: 2-7.
11. Wood PB. Mesolimbic dopaminergic mechanisms and pain control. Pain 2006; 120: 230-4.
12. Cury Y, Picolo G, Gutierrez VP, Ferreira SH. Pain and analgesia: the dual effect of nitric oxide in the nociceptive system. Nitric Oxide 2011; 25: 243-54.
13. Florentino IF, Galdino PM, De Oliveira LP, Silva DP, Pazini F, Vanderlinde FA, et al. Involvement of the NO/cGMP/KATP pathway in the antinociceptive effect of the new pyrazole 5-(1-(3-fluorophenyl)-1H-pyrazol-4-yl)-2H-tetrazole (LQFM-021). Nitric Oxide 2015; 47: 17-24.
14. Hajhashemi V, Salimian M, Hajihashemi O. Involvement of the NO/cGMP/K ATP pathway in the antinociceptive effect of rosemary (Rosmarinus officinalis) essential oil in mice. Behav Pharmacol 2023; 34: 37-44.
15. Pecikoza U, Micov A, Tomić M, Stepanović-Petrović R. Eslicarbazepine acetate reduces trigeminal nociception: possible role of adrenergic, cholinergic and opioid receptors. Life Sci 2018; 214: 167-75.
16. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992; 51: 5-17.
17. Hajhashemi V, Khodarahmi G, Asadi P, Rajabi H. Evaluation of the antinociceptive effects of a selection of triazine derivatives in mice. Korean J Pain 2022; 35: 440-6.
18. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol 2003; 225: 115-21.
19. Vazquez E, Navarro M, Salazar Y, Crespo G, Bruges G, Osorio C, et al. Systemic changes following carrageenan-induced paw inflammation in rats. Inflamm Res 2015; 64: 333-42.
20. Savegnago L, Jesse CR, Santos AR, Rocha JB, Nogueira CW. Mechanisms involved in the antinociceptive

effect caused by diphenyl diselenide in the formalin test. J Pharm Pharmacol 2008; 60: 1679-86.
21. Viguier F, Michot B, Hamon M, Bourgoin S. Multiple roles of serotonin in pain control mechanisms--implications of 5-HT_7 and other 5-HT receptor types. Eur J Pharmacol 2013; 716: 8-16.
22. Kapur S, Zipursky RB, Jones C, Wilson AA, DaSilva JD, Houle S. Cyproheptadine: a potent in vivo serotonin antagonist. Am J Psychiatry 1997; 154: 884.
23. Tan JQ, Fan CK, Cui J, Xu B. [Analgesic and antipyretic effects of cyproheptadine]. Zhongguo Yao Li Xue Bao 1990; 11: 204-7. Chinese.
24. Vale C, Oliveira F, Assunção J, Fontes-Ribeiro C, Pereira F. Co-administration of ondansetron decreases the analgesic efficacy of tramadol in humans. Pharmacology 2011; 88: 182-7.
25. Benarroch EE. Involvement of the nucleus accumbens and dopamine system in chronic pain. Neurology 2016; 87: 1720-6.
26. Wood PB. Role of central dopamine in pain and analgesia. Expert Rev Neurother 2008; 8: 781-97.
27. Morgan MJ, Franklin KB. Dopamine receptor subtypes and formalin test analgesia. Pharmacol Biochem Behav 1991; 40: 317-22.
28. Miclescu A, Gordh T. Nitric oxide and pain: 'something old, something new'. Acta Anaesthesiol Scand 2009; 53: 1107-20.
29. Kawabata A, Manabe S, Manabe Y, Takagi H. Effect of topical administration of L-arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. Br J Pharmacol 1994; 112: 547-50.
30. Bulutcu F, Dogrul A, Güç MO. The involvement of nitric oxide in the analgesic effects of ketamine. Life Sci 2002; 71: 841-53.
31. Ortiz MI, Granados-Soto V, Castañeda-Hernández G. The NO-cGMP-K+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin. Pharmacol Biochem Behav 2003; 76: 187-95.
32. Lázaro-Ibáñez GG, Torres-López JE, Granados-Soto V. Participation of the nitric oxide-cyclic GMP-ATP-sensitive K(+) channel pathway in the antinociceptive action of ketorolac. Eur J Pharmacol 2001; 426: 39-44.
33. Sakurada C, Sugiyama A, Nakayama M, Yonezawa A, Sakurada S, Tan-No K, et al. Antinociceptive effect of spinally injected L-NAME on the acute nociceptive response induced by low concentrations of formalin. Neurochem Int 2001; 38: 417-23.
34. Babbedge RC, Hart SL, Moore PK. Anti-nociceptive activity of nitric oxide synthase inhibitors in the mouse: dissociation between the effect of L-NAME and L-NMMA. J Pharm Pharmacol 1993; 45: 77-9.
35. Alves DP, Soares AC, Francischi JN, Castro MS, Perez AC, Duarte ID. Additive antinociceptive effect of the combination of diazoxide, an activator of ATP-sensitive K+ channels, and sodium nitroprusside and dibutyryl-cGMP. Eur J Pharmacol 2004; 489: 59-65.
36. Yamazumi I, Okuda T, Koga Y. Involvement of potassium channels in spinal antinociceptions induced by fentanyl, clonidine and bethanechol in rats. Jpn J Pharmacol 2001; 87: 268-76.
37. De Paz-Campos MA, Chávez-Piña AE, Ortiz MI, Castañeda-Hernández G. Evidence for the participation of ATP-sensitive potassium channels in the antinociceptive effect of curcumin. Korean J Pain 2012; 25: 221-7.
38. Makdissi A, Ghanim H, Vora M, Green K, Abuaysheh S, Chaudhuri A, et al. Sitagliptin exerts an antinflammatory action. J Clin Endocrinol Metab 2012; 97: 3333-41.