ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(2): 3429-3433
doi: 10.25083/rbl/27.2/3429.3433

Received for publication: May, 06, 2021
Accepted: May, 26, 2022

Original paper

The connection between nicotinic and adenosinic system in analgesia

CLAUDIA MARIANA HANDRA[1], ISABEL GHIȚĂ[2], DANIELA GEORGESCU[2], MARINELA CHIRILĂ[3]

[1]Department of Occupational Medicine, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, 8, Eroilor Sanitari Street, 050474, Bucharest, Romania
[2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, 8, Eroilor Sanitari Street, 050474, Bucharest, Romania
[3]„Titu Maiorescu University", Faculty of Pharmacy, Bucharest, Romania

Abstract

Determination of analgesic effects of different substances is important since this a very complex process that occur in the human body. The purpose of the present study was to evaluate the analgesic effect of caffeine and nicotine, both alone and in combination, using writhing test and hot plate test. The study used Swiss albino strain male mice, weighting 25 to 35 grams. The substances were administered subcutaneously. In the first experiments caffeine was administered in doses of 1 mg/kg and 5mg/kg and nicotine in doses of 1 mg/kg and 4 mg/kg. The last experiment evaluated the association between caffeine 5 mg/kg and nicotine 4 mg/kg. 30 minutes after administration, both doses of caffeine caused an antinociceptive effect, while nicotine induced analgesia only when the dose was increased to 4 mg/kg bw. Furthermore, when administered simultaneously, the two substances behaved like potentiating each other's effect, but additional research is needed in order to understand the mechanism of action.

Keywords Nicotine, caffeine, analgesia

To cite this article: HANDRA CM, GHIȚĂ I, GEORGESCU D, CHIRILĂ M. The connection between nicotinic and adenosinic system in analgesia. *Rom Biotechnol Lett.* 2022; 27(2): 3429-3433
DOI: 10.25083/rbl/27.2/429.3433

*Corresponding author: Assoc. Prof. Isabel Ghiță MD, PhD. Address: Blvd. Eroii Sanitari Nr. 8. Telephone: 0744887571. Email address: isabelghita@yahoo.co.uk

Introduction

Pain is defined, according to the International Association for the Study of Pain, as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [1]. Each individual describes pain differently, according to his past experiences and particular genetic background, but it is generally accepted that the human body uses this unpleasant sensation as an alarming signal to remove the injurious stimuli.

Physiologically, pain is classified as slow and fast pain, both categories serving different transmission pathways, but identical receptors, respectively the free nerve endings. One essential characteristic of nociceptive receptors is the lack of adaptation towards distress which massively influences the perception of pain.

Existing studies show that natural plant extracts are able to influence nociception, among other biological properties, like the antioxidative, anti-inflammatory, antiulcerogenic or immunomodulatory ones. Plantago species is a good example with its biologically active agents: flavonoids, iridoids, caffeic acid derivatives, polysaccharides, glycosides and terpenoids. Radu N and the team investigated the analgesic effects of various Plantago species. The study conducted of N. Radu et al. [2] proved the existence of a connection between polysaccharide extracts of Plantago species and pain perception; this study showed that polysaccharide extracts of Plantago species decreased analgesia. Further research showed that flavonoid extracts have no analgesic effects [3]. Moreover, a study that assessed the antinociceptive effect of iridoid extracts showed a partial analgesic response during writhing tests [4].

Caffeine is the most popular psychostimulant in the world, mostly consumed as coffee. It is a natural methylxanthine which stimulates the central nervous system, increases clinical alertness and generates restlessness by acting as a phosphodiesterase inhibitor, adenosine receptor antagonist and intracellular calcium modulator [5]. However, the biological effects of caffeine are predominantly provided by antagonizing adenosine receptors and affecting the adenosinergic tonus, which explains its effects when used acutely [6].

As mentioned above, caffeine inhibits adenosine receptors (A1, A2A, A2B, A3) which all show effects on spinal glial cells regarding the adjustment of nociception. However, recent studies indicate caffeine has more affinity at A1, A2A and A3 receptors compared to the A2B ones [7]. Even if adenosine receptors have similar distributions, they modulate nociception differently and, consequently, these complex mechanisms influence the way caffeine behaves towards analgesia. Nevertheless, recent data indicates occurrence of differences between genders that should be considered while assessing the efficacy of adenosine-based analgesics [7].

Nicotine, a highly addictive alkaloid found in tobacco plants, has both stimulatory and inhibitory effects on acetylcholine release. The mechanism of action depends on the administered dose and the predominance of either sympathetic or parasympathetic innervation of the selected organ system. Regarding the central nervous system (CNS), nicotine causes stimulation, thus improving concentration and reaction time. It diminishes stress and anxiety levels, while recent studies suggest nicotine could also have analgesic effects [8].

Several studies that investigated the role of nicotine in inducing antinociception by modulating the release of neurotransmitters, such as acetylcholine [8, 9, 10], glutamic acid [11] and other neuromodulators (dopamine, serotonin, noradrenaline) [8]. Hormonal signaling is implied as well, as in the case of corticotropin-releasing hormone [12].

Most importantly, nicotine, commonly consumed as cigarettes, is toxic and highly addictive, the addiction potential being influenced by genetic factors [13]. Considering these, nicotine is considered as it one of the most dangerous drugs available for everyday use.

Based on these literature data, our aim was to assess the way caffeine and nicotine affect analgesia and if both substances influence each other throughout the three experiments we conducted.

Material and methods

The analgesic effects of caffeine and nicotine were evaluated in three experiments on mice. The purpose of the first experiment was to study the sensitivity to pain 30 minutes after the administration of one dose of caffeine. The second experiment aimed to assess the analgesic effect of a single dose of nicotine 30 minutes after administration and the last experiment studied the identical behavior 30 minutes after administering simultaneously caffeine and nicotine.

The hot-plate and writhing tests were used to determine the analgesic effect of caffeine and nicotine. Pre-administered analgesic drugs, such as opioids, increase pain tolerance, the animal thus spending more time on the heated plate and writhing less compared to the control group.

3 groups of 12 albino male mice were used for conducting the experiments with caffeine and nicotine alone, while the third experiment, which combined both substances, used 4 groups of 12 albino male mice. All the animals weighted between 25 and 30 grams and were provided by the “Carol Davila” University of Medicine and

Pharmacy Bucharest bio-base. The mice were brought to the laboratory 24 hours prior to the start of the tests and were kept in standard environmental conditions with *ad libitum* access to food and water. The animals were housed in plexiglass cages (bed of wood chips), 12 mice per cage. The ambient temperature was set between 21°C and 24°C while the relative humidity was maintained between 45% and 60%.

The first experiment implied the administration of one dose of caffeine - 1 mg/kg bw or 5 mg/kg bw compared to the second experiment where nicotine was used in single doses of 1 mg/kg bw or 4 mg/kg bw. Finally, the third stage of our study combined caffeine 5 mg/kg bw and nicotine 4 mg/kg bw. Both substances were provided by Sigma Aldrich and were dissolved in 9‰ sodium chloride in order to administer 0.1 mL/10 g bw of solution. Additionally, all control groups received 0.1 mL/10 g bw of saline solution and each writhing test required 0.15 mL/10 g of acetic acid in concentration of 0.75%. The acetic acid was injected intraperitoneally while the other substances were administered subcutaneously.

All three experiments implied conducting hot-plate and writhing tests 30 minutes after injecting subcutaneously caffeine, nicotine or sodium chloride.

In the hot-plate test, the mouse is placed individually on a plate previously heated to 55°C and maintained at this temperature. Physiologically, after feeling the excessive heat, the animal starts licking its paw or attempts escaping by jumping on the side of the plate. The timer was started when all 4 paws of the mouse touched the plate and stopped when the animal began licking its paw. The cut-off time for the reaction was 30 seconds. In the writhing test, after injecting intraperitoneally an irritant such as acetic acid, the abdominal muscles start contracting and the animal acquire antalgic positions by pressing its back on the floor. We chose to measure the number of contortions performed in 5 minutes. If a substance increases the average amount of time spent on the hot-plate and decreases the number of contortions, compared to the control, it is considered to have analgesic effects.

The study was approved by the local ethics committee of "Carol Davila" University of Medicine and Pharmacy, Bucharest. The ethical agreement obtained was in concordance with the European Directive 86/609/EEC/24.11.1986 and with Governmental Decision 37/30.01.2002 referring to protection of experimental animals.

The obtained data was analyzed using *Microsoft Office-Excel*. Means and standard deviations were calculated for each group and then, the *Student-t test* was applied. Results were considered statistically significant if $p < 0.05$.

Results and Discussion

Evaluation of antinociceptive effect 30 minutes after administering caffeine

During the writhing test, the group that received 1 mg/kg bw of caffeine had a decreased number of contortions, with an average of 14.33 writhings, compared to the control group which presented an average of 22.25 ($p < 0.05$). The 5 mg/kg bw dose determined mice to behave similarly, the average number of contortions being 15.16, lower than the result of 22.25 obtained by the control group ($p < 0.05$) (Fig.1).

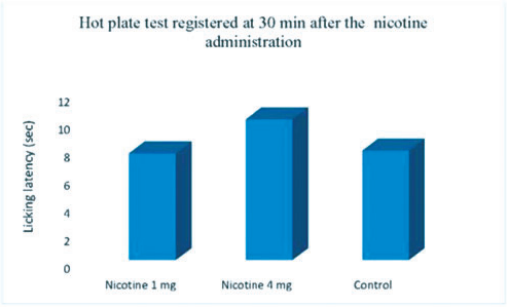

Figure 1. Writhing test: evaluation of antinociceptive effect 30 minutes after administering caffeine 1mg, 5mg and control.

In the hot-plate test, animals that were administered 1 mg/kg bw of caffeine spent, on average, 9.48 seconds on the heated surface, in contrast with the 7.88 seconds registered by those belonging to the control group ($p < 0.05$). Also, the dose of 5 mg/kg bw of caffeine caused an increased average amount of time, 9.52 seconds (Fig. 2), in comparison to control group's 7.88 seconds ($p < 0.05$).

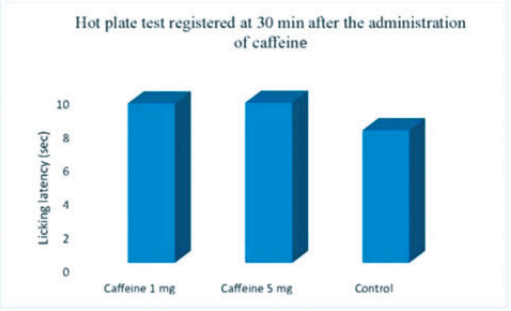

Figure 2. Hot-plate test: evaluation of antinociceptive effect 30 minutes after administering caffeine 1mg, 5mg and control

Evaluation of pain relief 30 minutes after administering nicotine

The writhing test conducted with the group which received 1 mg/kg bw of nicotine showed a diminished average number

of contortions, respectively 21.25, but the result is statistically insignificant considering the 22.5 average of the control group (Fig. 3). The batch injected with 4 mg/kg bw of nicotine performed, on average, 14.58 writhings, well below the average of the control group – 22.5 contortions ($p<0.05$).

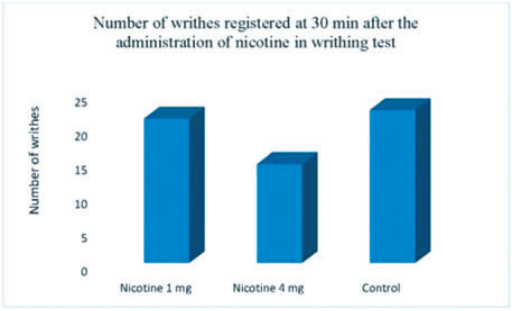

Figure 3. Writhing test: evaluation of antinociceptive effect 30 minutes after administering nicotine 1mg, 4mg and control

The hot-plate test revealed that 1 mg/kg bw of nicotine had induced a slight decrease - 7,64 seconds - in the average period of time the animals spent on the heated surface, which is statistically insignificant compared to the mean achieved by the control group – 7.84 seconds. In contrast, the mice treated with 4 mg/kg bw of nicotine (Fig. 4) resisted for an average of 10.08 seconds, far greater than the control group –7.84 seconds ($p < 0.05$).

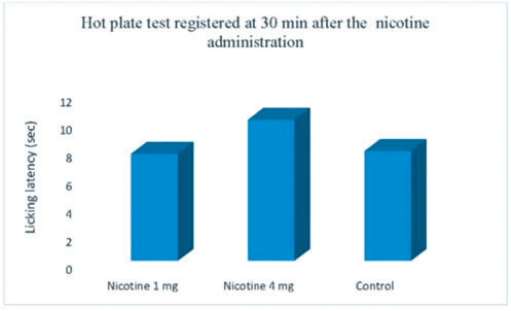

Figure 4. Hot-plate test: evaluation of antinociceptive effect 30 minutes after administering nicotine 1mg, 4mg and control

Evaluation of analgesia 30 minutes after administering caffeine and nicotine together

Regarding the writhing test, 5 mg/kg bw of caffeine determined an average number of 14.5 contortions, 4 mg/kg bw of nicotine obtained a mean of 13.5, while the control group recorded an average of 22.08 ($p < 0.05$ for each substance). Injecting simultaneously the previous doses of both drugs, the average number of contortions was 11.5 compared to the control group – 22.08 contortions ($p < 0.05$) (Fig. 5).

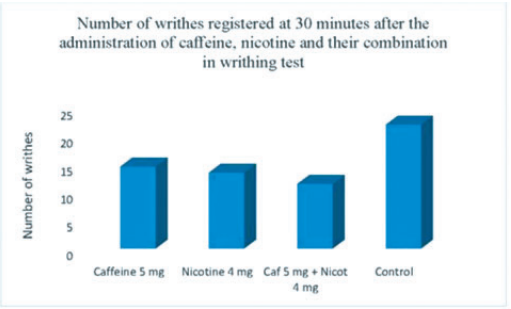

Figure 5. Writhing test: evaluation of analgesia 30 minutes after administering the combination of caffeine and nicotine

During the hot-plate test, 5 mg/kg bw of caffeine increased the average amount of time to 9.56 seconds, 4 mg/kg bw of nicotine did it to 10.37 seconds, while the control group managed to stay only 8.45 seconds on the plate ($p < 0.05$). As expected, combining the two substances led to a better outcome, which was 11.19 seconds, well above the average of 8.45 seconds achieved by the control ($p < 0.05$) (Fig. 6).

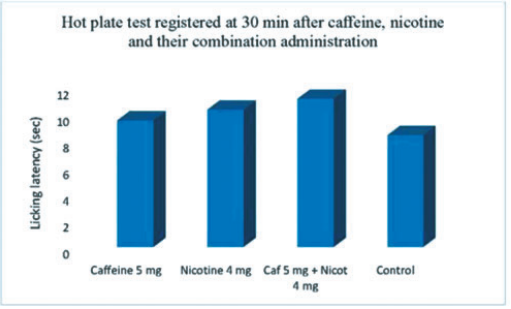

Figure 6. Hot-plate test: evaluation of analgesia 30 minutes after administering the combination of caffeine and nicotine

Conclusions

The results of the present study showed that, 30 minutes after administration, both doses of caffeine (1 mg/kg bw, 5 mg/kg bw) caused an antinociceptive effect by increasing pain tolerance throughout all experiments while only the high dose of 4mg/kg bw of nicotine induced analgesia. When administered simultaneously, the two substances behaved like potentiating each other's effect, but additional research is needed in order to understand the mechanism of action.

In addition, lower dose of caffeine proved to have similar or even better antinociceptive effect than the higher dose which could represent a starting point for further studies.

References

1. INTERNATIONAL ASSOCIATION FOR THE STUDY OF PAIN, https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698, accessed in 28.06.2020.
2. RADU N, GHITA I, RAU I. Therapeutic Effect of Polysaccharides from Plantago Species. *Mol. Cryst. Liq. Cryst.* 2010; 523: 236/[808]–246/[818].
3. NICOLETA R, GHITA I, COMAN O, RAU I. Therapeutic Effect of Flavonoids Derived from Plantago Species. *Mol. Cryst. Liq. Cryst.* 2010; 273/[845]–281/[853].
4. NICOLETA R, GHITA I, RAU I. Therapeutic Effect of Irridoidic Compounds from Plantago Species. *Mol. Cryst. Liq. Cryst.* 2010; 523: 289/[861]–296/[868].
5. LAMARINE RJ. Selected health and behavioral effects related to the use of caffeine. *J Community Health* 1994; 19 (6): 449-66.
6. VOICULESCU M, GHIȚĂ I, SEGĂRCEANU A, FULGA I, COMAN O. Molecular and pharmacodynamic interactions between caffeine and dopaminergic system. *Journal of Medicine and Life* 2014; 7(4): 30-38.
7. SAWYNOK J. Adenosine receptor targets for pain. *Neuroscience* 2016; 338: 1–18.
8. CHIRILA M, GHITA I, FULGA I. Current knowledge on bupropion and varenicline clinical efficacy in nicotine dependence. *Farmacia* 2015; (1):1-7.
9. DAMAJ MI, FONCK C, MARKS MJ. Genetic Approaches Identify Differential Roles for α4β2Nicotinic Receptors in Acute Models of Antinociception in Mice. *J Pharmacol Exp Ther* 2007; 321:1161-1169.
10. UMANA IC, DANIELE CA, MCGEHEE DS. Neuronal nicotinic receptors as analgesic targets: It's a winding road. *Biochem Pharmacol*. 2016; 86(8): 1208-1214.
11. SCHROEDER JA, QUICK KF, LANDRY PM. Glutamate transporter (GLT-1) activation enhances nicotine antinociception and attenuates nicotine analgesic tolerance. *Neuroreport* 2011; 22(18): 970-973.
12. BAIAMONTE BA, VALENZA M, ROLTSCH EA. Nicotine Dependence Produces Hyperalgesia: Role of Corticotropin-Releasing Factor-1 Receptors (CRF1Rs) in the Central Amygdala (CeA). *Neuropharmacology* 2014; 77: 217-223.
13. MARINELA CHIRILĂ, ISABEL GHIȚĂ, CLAUDIA MARIANA HANDRA, ION FULGA: Genetic variants influencing smoking behavior and efficacy of smoking
14. cessation therapies. *Romanian Biotechnological Letters Vol. 19, No. 5,2014*