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Original Research Article

To Evaluate the Acute and Chronic Effect of a Nicotine Administration, Effect of Its Abstinence Alone and Its Combination with Mecamylamine on Thermal Pain Perception and Body Weight of Albino Mice

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Abstract:

Introduction: Nicotine is a plant alkaloid and when given systemically, acts as an exogenous ligand of nicotinic acetylcholine receptor (nAChR). It is a tertiary amine that readily crosses the blood-brain barrier to bind to the central nAChR and nicotinic receptors on the ganglions.

Aim: we aimed to evaluate the acute and chronic effects of nicotine administration, the effect of abstinence alone, and its combination with mecamylamine on thermal pain perception and body weight in albino mice.

Materials & Methods: Animals were divided into four groups, each containing six mice. Group 1: treated with saline (subcutaneous). Group 2: nicotine 4 mg/kg/day administered in two daily subcutaneous injections for 7 and 14 days to analyze the acute and chronic effects on thermal pain sensation. Group 3: nicotine 4 mg/kg/day 2 daily subcutaneous injections for up to 7 days, then withdrawn. Group 4: nicotine 4 mg/kg/day 2 daily subcutaneous injections up to 7 days, then mecamylamine hydrochloride (2 mg/kg/day) in two doses on day 8. For the remaining 6 days (day 9 to14), the animals will receive only saline injections. All animals were fed a regular diet or high-fat diet. The amount of food consumed per mouse was recorded daily. The cumulative caloric intake was calculated based on the kcal/g of each diet. Each mouse was weighed at the start of the study (day 0) and subsequently every 3 days. Any weight change from baseline was noted.

Results & Conclusion: In addition to boosting reward- and antinociception-related brain systems, nicotine causes discomfort, paw withdrawal, and other unpleasant side effects. This study showed nicotine's unpleasant and antinociceptive effects, withdrawal, and neurobiology. Understanding the relationship between nicotine and tobacco usage may inspire new ways to quit.

Keywords: Nicotine; Nicotinic-acetylcholine-receptors, Mecamylamine Hydrochloride; Ganglions; Subcutaneous.

Introduction

Nicotine is a plant alkaloid and when given systemically, acts as an exogenous ligand of nicotinic acetylcholine receptor (nAChR). It is a tertiary amine that readily crosses the blood-brain barrier to bind to the central nAChR and nicotinic receptors on the ganglions. Nicotine stimulates the release of endogenous neurotransmitters, such as acetylcholine (ACh), dopamine, and endogenous opioid peptides derived from preproenkephalins, such as endorphins, enkephalins, and dynorphins [1,2]. These opioid derivatives contribute to the antinociceptive activity of nicotine by acting on the mu receptors [1].

Studies have shown that nicotine possesses acute antinociceptive effects when administered for a short period [3]. This was attributed to the release of opioid peptides. However, the effects of nicotine as an analgesic in chronic administration have been variable due to the subsequent development of tolerance [4,5]. Chronic use of nicotine reduces body weight and fat percentage in rodents fed a regular diet [6,7].

Mecamylamine is a non-selective, non-competitive, nicotinic receptor antagonist. It is a ganglion blocker that blocks synaptic transmission in the autonomic ganglia, skeletal neuromuscular junction, and central nervous system nicotinic synapses and is thus expected to alleviate the anti-nociceptive effects of nicotine. A previous study showed that nicotine withdrawal combined with

mecamylamine was more pro-nociceptive than nicotine withdrawal alone [3].

With this background, we aimed to evaluate the acute and chronic effects of nicotine administration, the effect of abstinence alone, and its combination with mecamylamine on thermal pain perception and body weight in albino mice.

Material and methods

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of the KIMS, Bhubaneswar. The present study was an observational study conducted with the aim of determining the acute and chronic effects of nicotine on albino mice. The primary endpoint was to study the antinociceptive effect of nicotine administration at the end of 7 and 14 days. The study also evaluated the effect of the nicotinic blocker mecamylamine on nociception after administration of nicotine for 7 days.

All procedures were conducted in accordance with Good Laboratory Practices and the Declaration of Helsinki. Adequate rehabilitation of experimental animals was ensured after the procedures and observations were performed.

Experimental animals

Albino mice weighing 20-25 gm were used to evaluate the effects of repeated nicotine administration. The mice were housed in polypropylene cages with 6/3 mice in the same group at the same temperature with a 12:12 light-dark cycle. The animals were fed a standard pellet diet and water ad libitum before and throughout the study period.

The animals were observed for a few days before starting the experiment. Healthy mice with normal behavior and activity were included in this study. Mice of both sexes were included in this study. Pregnant rodents and those weighing > 25 g were excluded from the study.

Drugs & treatment

Animals were divided into four groups, each containing six mice. Group 1: treated with saline (subcutaneous). Group 2: nicotine 4 mg/kg/day administered in two daily subcutaneous (s.c.) injections for 7 and 14 days to analyze the acute and chronic effects on thermal pain sensation. Group 3: nicotine 4 mg/kg/day 2 daily subcutaneous (s.c.) injections for up to 7 days, then withdrawn. Group 4: nicotine 4 mg/kg/day 2 daily subcutaneous injections up to 7 days, then mecamylamine hydrochloride (2 mg/kg/day) in two doses on day 8. For the remaining 6 days (day 9 to14), the animals will receive only saline injections. All animals were fed a regular diet or high-fat diet. The amount of food consumed per mouse was recorded daily. The cumulative caloric intake was calculated based on the kcal/g of each diet. Each mouse was weighed at the start of the study (day 0) and subsequently every 3 days. Any weight change from baseline was noted.

Nociceptive evaluation / evaluation of analgesia

The degree of antinociception was evaluated in terms of thermal pain using hot plate and tail-flick methods. These are the standard screening methods for the evaluation of central analgesics in rodents. Each mouse was observed for 6 s. The reaction time was recorded for each animal. The latency to paw withdrawal or tail flick was used to estimate the pain threshold. The animals were reused after proper rehabilitation in a central animal-house.

Observations and results:

A total of 24 mice were subjected to the drugs, and the aforementioned parameters were observed. The body weights of the mice in all four groups were measured at baseline (g). Consecutively, they were weighed every three days, and any change in weight in either direction was noted.

To evaluate antinociceptive parameters, the experimental animals were subjected to pain in the form of radiant and thermal heat. Two different methods i.e. tail flick analgesiometer for radiant heat and hot-plate method for thermal heat were used. The average tail flick latency (TFL) and paw withdrawal latency (PWL) periods (in seconds) were calculated at baseline and then on 8th day and 15th day subsequently. These were then compared to examine the acute and chronic effects of nicotine on the perception of nociceptive stimuli. Acute effects were evaluated after 7 days (i.e., on the 8th day), and chronic effects were evaluated after 14 days of nicotinic administration (i.e., on the 15th day).

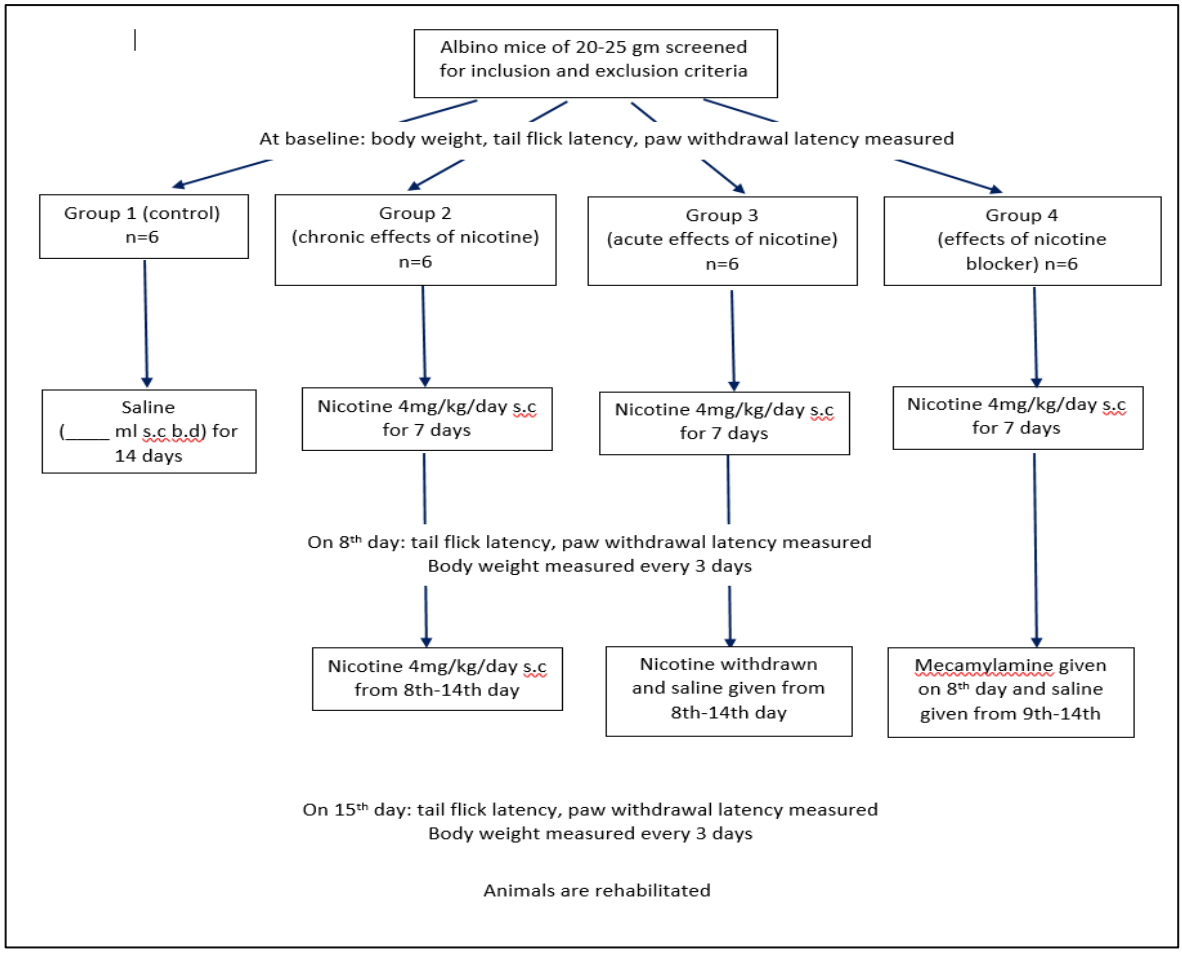

Figure 1: Flow diagram of the experiment

Table 1: Acute and chronic effects of nicotine on average weight of the experimental animals

	day1 (baseline)	day 4	day 7	p-value (ANOVA)	day 10	day 13	day 15	p-value (ANOVA)
Group 1	21.3	21.7	22.8	<0.05	23.5	23.8	24.0	➢ 0.05
Group 2	22.4	22.5	24.3		24.8	24.6	24.5	
Group 3	21.3	21.6	22.3		22.4	22.3	22.2	
Group 4	21.9	22.8	22.8		22.9	22.8	22.7	

Table 2: Acute and chronic effects of nicotine on tail flick latency (duration for 6 seconds) of the experimental animals using tail-flick analgesiometer

	baseline TFL	on 8th day	p-value (ANOVA)	on 15th day	p-value (ANOVA)
Group 1	2.52	2.49	< 0.05	2.3	< 0.05
Group 2	2.49	5.3		5.96	
Group 3	2.38	4.2		3.7	
Group 4	2.43	3.4		2.87	

Table 3: Acute and chronic effects of nicotine on paw withdrawal latency (duration for 6 seconds) of the experimental animals using hot-plate analgesiometer

	baseline PWL	on 8th day	p-value (ANOVA)	on 15th day	p-value (ANOVA)
Group 1	2.92	2.90	< 0.05	2.88	< 0.05
Group 2	2.88	4.65		5.43	
Group 3	2.90	4.13		3.81	
Group 4	2.84	4.22		3.12	

Discussion

Our results showed that nicotine affected pain sensitivity, leading to either a decrease or increase in paw withdrawal latency. When combined, the pronociceptive effects of mecamylamine hydrochloride prevailed over the antinociceptive effects of nicotine treatment, with mecamylamine hydrochloride animals displaying hyperalgesia regardless of previous repeated nicotine administration. Additionally, mecamylamine hydrochloride animals showed no change in weight in the study animals compared to all groups, with no significant difference compared to the other groups. The regression model demonstrated that repeated nicotine administration and higher concentrations of nicotine were independent predictors of a higher thermal pain threshold, while mecamylamine hydrochloride was associated with a > 50% decrease in pain threshold in the hot plate test. As part of the mesolimbic dopaminergic pathway, nucleus accumbens seems to mediate the reinforcing and aversive effects of nicotine and its withdrawal [16,17], as well as playing a role in pain modulation [18,19]. Nicotine has been shown to increase synaptic dopamine and D2 receptor sensitivity in the nucleus accumbens [20–22]. Both D1 and D2 dopamine receptors are expressed by neurons in the ventrolateral periaqueductal gray (vlPAG), and play a central role in morphine- and dopamine-induced antinociception [23,24]. The vlPAG acts as an output system responsible for integrating afferent inputs from multiple forebrain areas to modulate nociception in the spinal dorsal horn [25]. Recently, Umana et al. showed that 63% of the projections from the vlPAG to the rostral ventromedial medulla express α7 nicotinic acetylcholine receptors (nAChRs), suggesting a model of α7 nAChR-mediated analgesia in the vlPAG [26]. In their study, systemic and intra-vlPAG administration of an α7 nAChR-selective agonist showed an antinociceptive effect in the formalin assay, which was blocked by intra-vlPAG α7 antagonist pre-treatment [27]. Nicotine administration increased GABAergic transmission via presynaptic nAChRs in both α7-lacking and α7-expressing neurons in the vlPAG of rats [27]. Previous evidence indicates that nicotine might exert its antinociceptive effect mainly through the modulation of α4β2 nAChRs [6,28,29]. Activation of α4β2 nAChRs results in the stimulation of the dorsal raphe, nucleus raphe magnus, and locus coeruleus in a norepinephrine (NE)-dependent fashion [27–30]. These areas play a significant role in pain modulation through descending inhibitory pathways, which partly underlie nicotine-induced antinociception31. In addition, systemic administration of nicotine significantly increases the release of endogenous opioids, such as endorphins, enkephalins, and dynorphins, in the supraspinal cord via α7 nAChR30. Notably, the vlPAG descending pathway seems to be the key site by which mecamylamine hydrochloride increases nociceptive responses in rats. A recent study showed that mecamylamine hydrochloride decreased morphine-induced analgesia by modulating the vlPAG in rats [31]. Moreover, Sardi et al. [27] have shown that the nucleus accumbens also mediates the pronociceptive effect of mecamylamine hydrochloride through the activation of A2A adenosine receptors and inhibition of D2 dopamine receptors. In this study, an excitotoxic lesion of the nucleus accumbens prevented mecamylamine hydrochloride-induced hyperalgesia, which was reversed by acute blockade of this region through either an A2A adenosine antagonist or a D2 dopamine agonist [27]. Considering that A2A receptors are also largely involved in the homeostatic regulation of the sleep-wake cycle [32], we speculate that the role of nucleus accumbens A2A receptors in the pronociceptive effect of mecamylamine hydrochloride may also be linked to sleep pressure. The greater the sleep requirement, the greater the A2A receptor activity, and thus, the lower the pain threshold [27]. Considering that the vlPAG receives projections from nucleus accumbens neurons expressing A2A receptors, a possible integrative mechanism for the pronociceptive effects of mecamylamine hydrochloride could rely on the increased nucleus accumbens A2A activity, leading to the activation of the vlPAG descending pathway. Although no study has assessed the role of the nucleus accumbens adenosine receptors in pain processing, the use of adenosine receptor agonists has shown potent antinociceptive effects in animal models of chronic pain [36]. Spinal cord neurons expressing A2A adenosine receptors appear to mediate antinociception, inhibiting the symptoms of neuropathic pain [37].

In contrast, theophylline (an adenosine receptor antagonist) reduced nicotine-induced antinociception in a formalin test [38]. Taken together the evidence from the literature, we can speculate that in our study mecamylamine hydrochloride overcame the antinociceptive effects of nicotine pre-treatment by inhibiting the pain inhibitory descending vlPAG pathway through A2A receptor activation and D2 receptor inhibition in the nucleus accumbens27. It is possible that the antinociceptive effects of nicotine involve multiple pathways, including activation of α4β2 nAChRs29 and spinal A2A receptors36, release of opioids at the spinal cord30, and nucleus accumbens activation with projections to the vlPAG through α7 nAChR26. Pro-inflammatory cytokines, such as IL-6 and TNF-α, exert known pro-nociceptive effects through central and peripheral actions and are implicated in the pathophysiology of neuropathic pain39–42. We observed an increase in plasma concentrations of IL-6 when nicotine

abstinence and mecamylamine hydrochloride were combined. Nonetheless, there was no correlation between pain threshold and cytokines in the ABST-mecamylamine hydrochloride group, possibly due to the strong pro-nociceptive effect of mecamylamine hydrochloride itself, leading to a ceiling effect. However, when we adjusted the analysis of pain threshold for delta body weight, IL-6, IL-4, and TNF-α, the ABST-mecamylamine hydrochloride group showed the lowest latency to paw withdrawal, suggesting a synergistic effect of nicotine withdrawal and mecamylamine hydrochloride. Our findings point towards an inflammatory component in thermal sensitivity, as IL-4 was a predictor associated with pain threshold, although it did not differ statistically between the groups. With an observed OR of 1.006 and a mean concentration of IL-4 of 32.04 pg/mL, IL-4 may account for 21.13% of the thermal pain threshold variability in our sample. Thus, IL-4 may partially explain endogenous spontaneous individual differences in the pain threshold, independent of nicotine treatment or mecamylamine hydrochloride. Animal studies have shown that both IL-4 and IL-10 exert anti-nociceptive effects in various models of inflammatory pain; however, both cytokines were unable to increase the pain threshold in control animals43–45. Although IL-4 upregulates the expression of opioid receptors, the opioid antagonist naloxone does not reverse the antinociceptive effect of IL-4 in a model of inflammatory pain45.

Conclusion:

In addition to stimulating reward- and antinociception-related brain systems, nicotine induces aversive sensory effects such as pain, paw withdrawal, and other undesirable side effects. The competing aversive and antinociceptive effects of nicotine and its withdrawal, as well as its underlying neurobiology, were demonstrated in this study. Enhanced comprehension of the interplay between nicotine and tobacco use will, with luck, spark innovative strategies to reduce nicotine and tobacco consumption.

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