1. Introduction

In the central nervous system, acetylcholine (ACh) acts on the nicotinic acetylcholine receptor (nAChR) and regulates numerous cellular functions, including neurotransmitter release, cell excitability, and neuronal integration. The nAChRs play a crucial role in neurophysiological functions, such as cognition, reward, motor activity, and analgesia, and in pathological conditions, such as Alzheimer’s disease, Parkinson’s disease, some forms of epilepsy, depression, autism, and schizophrenia (1). The nAChR family consists of ligand-gated cation channels with a pentameric structure composed of α and β subunits, and there are several subtypes of these channels based on subunit configuration (1, 2). Nicotine (NIC) is an exogenous ligand of these nAChRs and influences various physiological functions.

NIC activates nAChRs located on neurons in the peripheral and central nervous system and thereby exerts numerous acute effects, such as antinociception and activation of the hypothalamic–pituitary-adrenal (HPA) axis [i.e., corticosterone (CORT) increase in rodents], in addition to diarrhea, miosis, nausea, vomiting, blood pressure elevation, amnesia, tremor, and convulsion. Moreover, there is wide evidence that an opioid-receptor antagonist, naloxone (NLX), prevents the NIC-induced antinociception in mice (3, 4), indicating the participation of the endogenous opioid system in NIC-induced antinociception. However, there is no report evaluating the relationship between other NIC effects and endogenous opioid system. Besides, NIC has chronic effects as well, including psychological and physical dependence liability. NIC is usually ingested via tobacco smoking, and it was reported that most heroin users under opioid substitution therapy were habitual smokers (97.7% of heroin users) (5). Furthermore, compared with the general population, heroin users showed extremely high...
rates of tobacco smoking (6, 7), and heroin abstinence after detoxification had been associated with increased smoking consumption (7). These results indicate that the endogenous opioid system stimulated by NIC may alleviate opioid abstinence. However, there are few reports indicating the involvement of NIC-induced activation of the endogenous opioid system in NIC dependence. In this review, we discuss the involvement of the endogenous opioid system in the acute and chronic effects of NIC and the relationship between nAChR subtypes and the opioid system, particularly in reference to our recent research.

2. NIC and endogenous opioid peptides

It is well known that NIC elicits the release of neurotransmitters, such as norepinephrine, dopamine, acetylcholine, serotonin, glutamate, and GABA (2). The activation of nAChR by NIC leads to a conformational change in the central pore, which results in the influx of sodium and calcium ions. This influx of cations depolarizes nerve endings and stimulates calcium influx through voltage-dependent N-type calcium channels, thereby triggering exocytotic neurotransmitter release (8). As for neurotransmitter release, there are several lines of evidence indicating that NIC induces the release of endogenous opioid peptides (Table 1). For example, acute NIC administration (1 mg/kg, s.c., 1 h later) decreases β-endorphin content in the hypothalamus evaluated by radioimmunoassay. The mRNA expression of proopiomelanocortin (a precursor of β-endorphin) in the hypothalamus was unchanged following acute NIC (9). The authors interpreted the events as enhanced release and degradation of the opioid peptide. The hypothalamus is the principal site of β-endorphin-producing cells in the brain, along with the endorphinergic terminal fields of the striatum and hippocampus. β-Endorphin immunoreactivity is increased in the conditioned medium of primary fetal hypothalamic cell cultures exposed to NIC (6 – 18 μM) for 6 h (10). Acute NIC administration (0.3 mg/kg, s.c.) increases Tyr-Gly-Gly [an extraneuronal metabolite of opioid peptides derived from proenkephalin (PENK) A] levels in the nucleus accumbens and in the lower brain stem, including the dorsal raphe, pontine reticular formation, gigantocellular reticular formation, locus coeruleus, sensory trigeminal nucleus, and the caudal part of the ventrolateral medulla oblongata. Concomitantly, NIC produces a significant decrease in

| Table 1. Nicotine effects on endogenous opioid peptides |
|-----------------|-----------------|-------------|------------|
| Treatment       | Changes of content | Brain region          | Ref. No |
| ENDOPHINS       |                  |                        |          |
| NIC 1 mg/kg, s.c. in vivo | β-endorphin ↓                  | Hypothalamus                        | (9)     |
|                  |                   | Striatum                        |          |
|                  |                   | Hippocampus                       |          |
|                  |                   | Prefrontal cortex                 |          |
| POMC mRNA →      |                   | Hypothalamus                        |          |
|                  |                   | Prefrontal cortex                 |          |
| NIC 6-18 μM in vitro | β-endorphin ↑                  | In conditioned medium             | (10)    |
| ENKEPHALINS      |                  |                        |          |
| NIC 0.3 mg/kg, s.c. in vivo | Tyr-Gly-Gly ↑                  | Nucleus accumbens                 | (11)    |
|                  | Extraneuronal metabolites   | Lower brain stem                  |          |
|                  | Met-enkephalin ↓                  | Amygdala                          |          |
|                  |                                 | Cerebellum                        |          |
|                  |                                 | Medulla                            |          |
|                  |                                 | Spinal cord                       |          |
| DYNORPHINS       |                  |                        |          |
| NIC 1 mg/kg, s.c. in vivo | Dynorphin 1-13 ↑                | Striatum                          | (12)    |
|                  | biphasic pattern              | Caudate putamen                   |          |
|                  |                                 | Nucleus accumbens                 |          |
|                  |                                 | Hypocampus                        |          |
|                  |                                 | Hypothalamus                       |          |
| Prodynorphin mRNA ↑ | prolonged time               | Caudate putamen                   |          |
|                  |                                 | Nucleus accumbens                 |          |

These references indicate the enhanced release and degradation of opioid peptides. Abbreviations: NIC, nicotine; POMC, proopiomelanocortin; Tyr, tyrosine; Gly, glycine. ↑: Increase, ↓: Decrease, →: No change.
native methionine-enkephalin (Met-Enk) levels in the central amygdala, flocculonodular lobe of the cerebellum, caudal part of the ventrolateral medulla, and the intermediolateral cell column of the spinal cord, reflecting release of the opioid peptide at these sites (11). Furthermore, Isola et al. (12) reported that acute NIC (1 mg/kg, s.c.) induces an increase in striatal dynorphin 1-13 content and that prodynorphin mRNA levels were elevated in the striatum for a prolonged time (0.5 – 24 h). The content of dynorphin 1-13 increased soon after the rise of prodynorphin mRNA, at 1 h after NIC. The increment of dynorphin 1-13 content displayed a biphasic pattern; at 1 h after NIC, the content of dynorphin 1-13 was increased, but it declined to near the control level between 2 and 4 h. Subsequently, dynorphin 1-13 content rose again by 6 h, reached maximal levels at 12 – 24 h, and approached the control level by 48 h. The authors interpreted this biphasic pattern of dynorphin 1-13 content as accelerated dynorphin 1-13 release and subsequent breakdown resulting in lower content. Taken together, these findings suggest that some of the physiological and pharmacological effects of NIC may be mediated by endogenous opioid peptides.

Kiguchi et al. (13) reported that PENK mRNA levels were significantly increased in the lumbar spinal cord, but not the dorsal root ganglia, 2 h after NIC administration (5 mg/kg, s.c.), declining to control levels at 4 h. This increase in PENK mRNA levels was inhibited by the nAChR antagonist, mecamylamine (3 mg/kg, s.c., 15 min before NIC). However, mRNA levels of proopiomelanocortin and prodynorphin were not increased by NIC administration. In the dorsal horn of the lumbar spinal cord, Met-Enk immunoreactivity was remarkably reduced at 0.5 h following NIC administration, by immunohistochemistry, indicating release of Met-Enk from presynaptic terminals. Met-Enk immunoreactivity returned to control levels 2 h after NIC administration. These results suggest that NIC also promotes the release of opioid peptide and activates the endogenous opioid system in the spinal cord.

3. Acute effects of NIC

3.1. Comparison of NIC effects associated with or without endogenous opioid system

Pain-alleviating effects: Pain is classified into three types: nociceptive pain, neurogenic pain, and psychogenic pain. Analgesic agents can alleviate nociceptive and neuropathic pain, although neurogenic pain is sometimes resistant to treatment with analgesic agents such as opioid analgesics and non-steroidal anti-inflammatory drugs.

Nociceptive pain is elicited by the activation of nociceptors located on peripheral nerve endings by mechanical, thermal, or chemical stimuli. Systemic administration of NIC suppresses nociceptive pain elicited by thermal and mechanical noxious stimuli in rodents, as assessed with the tail-flick test (14), tail-immersion test (15, 16), hot-plate test (14, 15, 17), and tail pinch test (13, 18). Microinjection of an nAChR agonist (ABT-594) into the nucleus raphe magnus (19) and intrathecal administration of NIC (13) showed antinociceptive effects by the hot plate test and tail pinch test, respectively. Systemic NIC-induced antinociception is antagonized not only by the nAChR antagonist mecamylamine, but also by the opioid-receptor antagonist NLX (13, 17, 18), as shown in Table 2A. Additionally, NIC- and morphine-induced antinociception is reduced in morphine-tolerated mice (40 mg/kg, s.c., twice a day for 4 days) in comparison with naïve mice (18). Moreover, it has been reported that NIC is less potent in reducing pain in μ-opioid receptor gene–knock-out mice compared with wild-type mice (20). Furthermore, NIC is less potent in producing antinociception in PENK gene–knock-out mice than in wild-type mice (15), indicating that endogenous opioid peptides derived from PENK are involved in NIC-mediated antinociception. Collectively, these findings demonstrate that the endogenous opioid system may play an important role in mediating the antinociceptive effects of NIC.

Neuropathic pain is a form of neurogenic pain characterized by allodynia, hyperalgesia, and spontaneous pain. Neuropathic pain occurs after injury to the central or peripheral nervous system and is mediated by neuro-inflammation following nerve injury. There is growing evidence suggesting that the NIC–nAChR system acts as a negative regulator of inflammation. Indeed, systemic administration of NIC (1.5 mg/kg, i.p.) suppresses neuropathic pain induced by antiviral and anticancer agents (dideoxycytidine and oxaliplatin, respectively), as well as that elicited by chronic constriction injury of the sciatic nerve in rats (21). Moreover, intrathecal administration of NIC or epibatidine (an nAChR agonist) reversed the thermal and mechanical hyperalgesia in mice that received partial sciatic nerve ligation (PSL) (22).

It is generally assumed that immune cells act predominantly as generators of neuropathic pain and that neuro-inflammation is involved in this process (23 – 26). Peripheral tissue injury causes the migration of immune cells to the injured site (27). These cells express opioid peptides, such as β-endorphin, Met-Enk, and dynorphin A, together with corticotropin-releasing factor (CRF) receptor. Local administration of CRF potently inhibited the nociception in inflamed tissue accompanied by opioid peptides release, which were antagonized by co-administration of a CRF antagonist, a-herical CRF (28 – 30). Importantly, CRF-induced release of β-
endorphin from immune cells was calcium-dependent and was evoked by increasing potassium concentration, suggesting that opioid peptides were released from vesicles, similar to the situation in neurons (29). Thus, selective stimulation of these immune cells by local administration of CRF elicits opioid peptide release, resulting in the inhibition of tactile allodynia in mice subjected to sciatic nerve injury (31). Kiguchi et al. (32) reported that protein levels of the nAChR $\alpha_4$ and $\alpha_7$ subunits were upregulated following PSL. Immuno-reactivity for the $\alpha_4$ and $\alpha_7$ subunits was localized on both bone marrow–derived macrophages and neutrophils in the injured nerve. PSL-induced tactile allodynia and thermal hyperalgesia are significantly alleviated by perineural (local) administration of NIC (20 nmol, once a day for 4 days after PSL), and this effect of NIC can be blocked by concomitant administration of mecamylamine. Given that NIC elicits the release of endogenous opioid peptides in the central nervous system, these results suggest that locally applied NIC alleviates neuropathic pain by inducing the release of opioid peptides from immune cells. However, the effects of the locally administered NIC on PSL-induced neuropathic pain persisted for at least 11 days after the last NIC treatment (32). Thus, further study is required to clarify the role of immune cell–derived opioid peptides in NIC-induced suppressive effect of neuropathic pain and to elucidate the underlying mechanisms.

**HPA axis activating effects:** NIC elevates levels of the circulating stress-response hormone CORT by activating the HPA axis in rodents (18, 33, 34). NIC acts on the nucleus tractus solitarius, and noradrenaline is released in the hypothalamic paraventricular nucleus, resulting in the stimulation of CRF release. CRF acts on the pituitary, inducing the secretion of adrenocorticotropic hormone, which activates the synthesis and secretion of CORT in the zona fasciculata of the adrenal cortex (35). Yamamoto et al. (18) reported that a single administration of NIC increases CORT levels in mice in a dose-dependent manner. The NIC-induced elevation in CORT levels was inhibited by mecamylamine (an nAChR antagonist), but not by NLX (an opioid-receptor antagonist). Moreover, the NIC-induced increase in CORT levels was not reduced in morphine-tolerated mice (40 mg/kg, s.c., twice a day for 4 days), suggesting that the endogenous opioid system may not participate in the NIC-induced activation of the HPA axis (Table 2A). These results are consistent with the report of Mellon and Bayer (33) showing that, in rats, naltrexone (NTX), an opioid-receptor antagonist, does not block the epibatidine-induced increase in CORT levels, although it suppresses the morphine-induced increase in CORT.

### 3.2. The NIC effects and nAChR subtypes

The nAChR family consists of homomeric and heteromeric complexes of protein subunits ($\alpha_{2-10}$ and $\beta_{2-4}$) in the central nervous system (1, 2). In agreement with in situ hybridization studies showing the widespread distribution of $\alpha_4$-, $\beta_2$-, and $\alpha_7$-subunit mRNAs in the brain; $\alpha_7$-subunit–containing nAChRs; and $\alpha_4$- + $\beta_2$-

Table 2. Summary of the impact of opioid receptor and nAChR antagonists on the NIC effects

<table>
<thead>
<tr>
<th>A. Acute effects of nicotine (see ref. 18)</th>
<th>Antinociception</th>
<th>CORT elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>naloxone (NLX)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>mecamylamine</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>Tolerance to morphine</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>dihydro-β-erythroidine (DH/E)</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>methyllycaconitine (MLA)</td>
<td>→</td>
<td>↓</td>
</tr>
</tbody>
</table>

↓: antagonism or suppression
→: no effect

<table>
<thead>
<tr>
<th>B. Chronic effects of nicotine (see ref. 49)</th>
<th>Naloxone-precipitated withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>naltrexone (NTX)</td>
<td>—</td>
</tr>
<tr>
<td>dihydro-β-erythroidine (DH/E)</td>
<td>+</td>
</tr>
<tr>
<td>methyllycaconitine (MLA)</td>
<td>—</td>
</tr>
</tbody>
</table>
subunit–containing nAChRs are the most abundantly expressed subtypes, although their regional expression varies in different vertebrate species (1). These receptors are expressed in several brain regions associated with pain transmission and activation of the HPA axis in rodents.

It has been reported that NIC-induced antinociception is mediated specifically by α7 and α4β2 nAChR subtypes (36–38), consistent with a previous report showing that NIC-induced antinociception is suppressed by both methyllycaconitine (MLA), an antagonist of the α7 nAChR, and dihydro-β-erythroidine (DhβE), an antagonist of the α4β2 nAChR, in mice, as evaluated using the tail-pinch test (18). In comparison, the NIC-induced increase in CORT levels was suppressed by DHβE, but not by MLA (ref. 18, Table 2A). These findings suggest that the α4β2 nAChR plays an important role in both NIC-induced antinociception and CORT increase, while the α7 nAChR participates in NIC-induced antinociception, but not in CORT increase. Although NIC-induced antinociception was mediated by the endogenous opioid system, the NIC-induced increase in CORT levels was not. According to these results, the authors concluded that the endogenous opioid system was not located in the down-stream of α4β2 nAChR and postulated that the opioid system might be located in the down-stream of α7 nAChR (18).

4. Chronic effects of NIC

4.1. Psychological dependence

Psychological dependence on NIC is characterized by craving and reward behavior in mammals. The rewarding effects of NIC manifest as self-administration and conditioned place preference (CPP) in experimental animals. Recent evidence suggests that activation of stress circuits, including the dynorphin/κ-opioid-receptor system, modulates the rewarding effects of addictive drugs such as NIC. Activation of the κ-receptor, either by repeated forced swim stress or administration of U50,488 (5 or 10 mg/kg, i.p.), significantly potentiates NIC-evoked CPP. This potentiation of NIC-evoked CPP was blocked by the systemic injection (10 mg/kg, i.p.) or local injection in the amygdala (2.5 μg) of the κ-opioid–receptor antagonist nor-binaltorphimine, without affecting NIC reward in the absence of stress. These results indicate that the activation of the dynorphin/κ-opioid system in the amygdala may be involved in the potentiation of the NIC reward effect, i.e., the development of psychological dependence (39). In fact, it is reported that acute psychosocial stress increased cigarette craving in smokers (40). Conversely, the average number of exposure to a traumatic event (i.e., involving serious body injury, threat of death, or witnessing violent death or injury) was significantly higher in individuals with NIC-dependence than that in non-NIC dependent individuals (41). These reports suggest that the stressful events elevate the risk for NIC-dependence in humans.

Human obese smokers self-administered NIC via cigarettes significantly less often than non-obese smokers, and the hedonic effects of NIC-containing cigarettes were attenuated in obese smokers compared to non-obese smokers (42). Similarly, mice exposed to a high-fat diet exhibited lower levels of NIC-evoked CPP, relative to control mice. mRNA levels of μ-opioid and leptin receptors were also downregulated in the ventral tegmental area of these obese mice (42). This study may provide evidence of reduced NIC reward in obese subjects and suggests that this reduced NIC reward in obesity may be mediated by dietary influences on the endogenous opioid system. However, further study is required to clarify the relationship between obesity and NIC reward through the endogenous opioid system.

4.2. Physical dependence

Physical dependence on NIC is characterized by withdrawal signs, including irritability, anxiety, inability to concentrate, and insomnia in humans (43), and by teeth chattering, chewing, grasping, writhing, body shakes, ptosis, and seminal ejaculation in experimental animals (44). The withdrawal signs of NIC closely resemble the commonly observed withdrawal signs of opioids (44). It has been reported that an increase in CORT levels is one of the signs of morphine withdrawal and that the magnitude of the increase is an effective, objective, and quantitative indicator of spontaneous and opioid-receptor antagonist–precipitated morphine withdrawal in rodents (45, 46). The CORT increase elicited by morphine withdrawal is caused by the activation of A2 adrenergic cells in the nucleus of the solitary tract (47), which is mechanistically similar to the NIC-induced activation of the HPA axis (35).

There is accumulating evidence indicating that NIC-induced activation of the endogenous opioid system may be involved in the development of physical dependence. For example, the opioid-receptor antagonist NLX precipitates NIC abstinence syndrome in the rat (48), and the development of dependence on NIC is attenuated in μ-opioid-receptor–knockout mice (20). If physical dependence on NIC develops through the activation of the endogenous opioid system, NLX may precipitate NIC withdrawal. Thus, signs of opioid withdrawal, including CORT increase, might be useful for evaluating NLX-precipitated NIC withdrawal. Ueno et al. (49) reported that NLX elicited an increase in CORT levels in mice receiving repeated NIC administration, and the
magnitude of the increase was dependent on the dose and number of days of NIC treatment. Moreover, NTX (an opioid-receptor antagonist), when concomitantly administered with NIC, inhibited the NLX-induced CORT increase in a dose-dependent manner, indicating that NTX suppresses the development of physical dependence on NIC (Table 2B). These results suggest that the development of physical dependence on NIC is mediated by activation of the endogenous opioid system.

Yamamoto et al. (18) suggested that the effects of NIC on the endogenous opioid system might be mediated by a non-α4β2 subtype of nAChR. Ueno et al. (49) further clarified the relationship between nAChR subtype and the endogenous opioid system in mice with physical dependence on NIC. In their experiment, concomitant administration of the α7 nAChR antagonist MLA with repeated NIC treatment blocked NLX-induced CORT increase, indicating the inhibition of the development of physical dependence on NIC. However, an NLX-induced increase in CORT levels was observed when the α4β2 nAChR antagonist DHβE was concomitantly administered with repeated NIC, demonstrating the development of physical dependence on NIC (Table 2B). These results strongly suggest that the effects of NIC on the endogenous opioid system that result in the development of physical dependence are mediated by activation of the α7 nAChR.

5. Conclusion and perspective

NIC poses two major problems in clinical medicine. First, accidental ingestion of tobacco frequently occurs in children and can elicit severe NIC toxicity. Second, NIC makes it difficult to cease tobacco smoking because of its dependence liability. Repeated exposure to tobacco smoke can lead to numerous diseases, including neoplastic, pulmonary, and cardiovascular diseases, and it continues to be the leading cause of preventable deaths (50). Through repeated intake of NIC via tobacco smoking, psychological and physical dependence develop. Psychological dependence manifests as craving behavior for NIC, leading to frequent intake of the drug. Once physical dependence develops, withdrawal symptoms are elicited following cessation of NIC intake. Consequently, many smokers relapse to overcome NIC withdrawal. Thus, it is important to clarify the mechanisms underlying the development of NIC dependence.

In this review, we discussed the involvement of the endogenous opioid system in mediating the effects of NIC. Specifically, we focused on the accumulating evidence suggesting that the development of physical dependence on NIC is mediated by the endogenous opioid system located on the downstream of the α7 nAChR, but not the α4β2 nAChR. The importance of the endogenous opioid system in NIC-mediated physical dependence suggests that opioid-receptor antagonists may be a useful agent as the adjunctive therapy for smoking cessation, for example, the maintenance therapy after tobacco use cessation by means of inhibition of the development of physical dependence due to resumption of tobacco smoking.

With regard to α4β2 nAChR, NIC reinforcement was eliminated by disruption of the nAChR β2-subunit gene in mice and was restored by reinserting the gene into the ventral tegmental area of the midbrain (51). NIC activates α4β2 nAChR in the ventral tegmental area, resulting in dopamine release in the shell of the nucleus accumbens, whereas the dopaminergic neurons in these brain regions are critical in drug-induced reward (52). These facts suggest that α4β2 nAChR may play an important role in psychological dependence. Hence, despite the critical importance of the α7 nAChR, varenicline (a partial agonist of the α4β2 nAChR) has been used to aid smoking cessation (53, 54). On the other hand, spontaneous NIC withdrawal (somatic withdrawal behaviors) occurs in α7 nAChR–knockout mice with physical dependence induced by chronic exposure to NIC in the drinking water (55), suggesting that α4β2 nAChR may be involved in the development of physical dependence on NIC via a non-opioid mechanism. Therefore, further research is required to clarify the role of the α4β2 nAChR and non-opioid systems in the development of NIC dependence.

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References


