The effect of nicotine on antidepressant and anxiolytic responses induced by citalopram and citicoline in mice

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The effect of nicotine on both anxiety and depression has been broadly studied. Moreover, citalopram and citicoline play a role in the modulation of anxiety and depression. This study was designed to examine the effects of nicotine on the antidepressant and anxiolytic responses induced by citalopram and citicoline in mice. Anxiety- and depression-related behaviors were assessed with the elevated plus maze and forced swim test, respectively. The results showed that subcutaneous administration of nicotine decreased open-arm time (OAT) and open-arm entries (OAE) but increased immobility time, suggesting anxiogenic-like and depressive-like effects. Intraperitoneal administration of citalopram increased OAT but decreased immobility time, indicating that citalopram induced anxiolytic-like and antidepressant-like responses. Additionally, an injection of citicoline increased OAE but decreased immobility time, revealing anxiolytic-like and antidepressant-like effects. Interestingly, the subthreshold dose of nicotine potentiated the citalopram and citicoline effects on OAT and immobility time, which revealed anxiolytic-like and antidepressant-like behaviors. Locomotor activity was not significantly changed by any doses of the drugs. In conclusion, these findings suggest that interactions between nicotine and citalopram or citicoline occur upon induction of anxiolytic and antidepressant responses in mice.

Key words: nicotine, citalopram, citicoline, depression, anxiety

INTRODUCTION

Nicotine, as the main component of tobacco, is involved in many processes in both humans and animals, such as anxiety, depression, reward, and addiction (Picciotto et al., 2002; 2015; Andreasen and Redrobe, 2009; Zarrindast and Khakpai, 2019). Nicotine exerts its effect by activating nicotinic acetylcholine receptors (nAChRs), which are highly expressed throughout the brain. These receptors are ligand-gated ion channels and have 12 diverse neuronal nAChR subunits, including α2–α10 and β2–β4 (Zarrindast and Khakpai, 2019). The nAChRs in the nervous system are principally located presynaptically, where they modulate the release of various neurotransmitters, including acetylcholine (ACh), serotonin (5-HT), and norepinephrine (NE) (Kenny et al., 2001). Increased neurotransmitter release, acting at numerous postsynaptic receptors, is the normal mechanism through which nicotine modulates behaviors (Wonnacott, 1997). Of these postsynaptic receptors, the 5-HT receptor plays the primary role in mediating nicotine-induced responses (File et al., 2000; Kenny et al., 2001). Studies have revealed that nicotine administration can increase transcription of the 5-HT1A receptor and increase 5-HT signaling (Kenny et al., 2001; Picciotto et al., 2002). Any interaction between nicotine and the modulation of 5-HT receptor gene expression is of clinical importance, given the key role of these receptors in anxi-
The effect of nicotine on antidepressant activity and depression (Haddjeri et al., 1998; Heisler et al., 1998; Kenny et al., 2001).

The 5-HT neurons project from the raphe nuclei throughout the brain, where they modulate many functions, e.g., sleep, depression, and anxiety (Silber and Schmitt, 2010; Sprowles et al., 2016). Synaptic concentrations of 5-HT are regulated by the serotonin reuptake transporter (SERT). Selective serotonin reuptake inhibitors (SSRIs), such as citalopram, block the SERT and increase synaptic 5-HT (Sprowles et al., 2016), hence modulating depression and anxiety disorders (Varia et al., 2002; Kokras et al., 2011; Cui et al., 2018; Arias et al., 2019). Citalopram acts as a non-competitive antagonist of several nAChRs. The most compelling evidence that citalopram modulates nAChR activity is based on animal research showing that non-selective (e.g., nicotine) and α7-selective (e.g., PNU-282987) agonists increase the activity of this antidepressant (Popik et al., 2003; Andreasen et al., 2011; Arias et al., 2019).

Moreover, there is an interaction between citalopram and citicoline (cytidine-5’-phosphocholine) in the modulation of anxiety and depression. Research reports indicate that citicoline, in combination with citalopram, increased the effectiveness of SSRI drugs for depression and anxiety treatment (Roohi-Azizi et al., 2017; 2018; Nejati et al., 2020). Several studies revealed that citicoline was an effective therapeutic treatment for managing post-stroke patients suffering from anxiety or anxious-depressive syndrome (Corallo et al., 2020; Arcadi et al., 2021). Citicoline is used in the treatment of neurodegenerative diseases such as ischemia, dementia, and addictive disorders (Adibhatla and Hatcher, 2005; Gruber et al., 2015), and it may induce antidepressant and anxiolytic effects by increasing monoaminergic neurotransmitters (Yoon et al., 2010; Abdolmaleki et al., 2016; Roohi-Azizi et al., 2018). Based on the interactions between nicotine and 5-HT receptors (Kenny et al., 2001), citalopram and nAChRs (Arias et al., 2019), as well as citalopram and citicoline (Roohi-Azizi et al., 2018), we chose to determine whether a subthreshold dose of nicotine has an effect on anxiolytic-like and antidepressant-like responses induced by citalopram and citicoline in mice.

METHODS

Animals

Male NMRI mice (20–25 g) obtained from the Tehran University of Medical Sciences (Tehran, Iran) were used for all experiments and were 6–8 weeks of age at the time of testing. NMRI mice were kept in an animal house with a 12 h light/dark cycle, a controlled temperature of 22 ± 2°C, and 55 ± 10% relative humidity. Mice were housed eight per cage in Plexiglas cages. Food and water were available ad libitum. Experiments were performed between 8:00 a.m. and 2:00 p.m. Each mouse was used only once. All experiments were approved by the Research and Ethics Committee of Tehran University of Medical Sciences. They were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23).

Elevated plus-maze (EPM)

All tests were performed in a dimly lighted room, separated from the colony room. Mice (n=8) were adapted to the test room for 1 h before the experimental test. The EPM apparatus consisted of two open arms (40 × 7) and two closed arms of similar size plus 10 cm-high end and side walls. The arms were linked via a central 7 cm × 7 cm area. A mouse was placed in the center of the apparatus with its head facing an open arm and left undisturbed for 300 sec. Then, the mouse was removed and returned to its home cage. The apparatus was cleaned with a 10% chlorine bleach solution between individual test sessions. The mouse was considered to be on the central platform when at least two paws were on it and in an arm whenever all four paws were on it. The time spent in open arms [OAT: (time in open arm/time in open + closed arm)] and the open arm entries [OAE: (number of open arm entries/number of open + closed arm entries)] were used as a measure for anxiety. Moreover, the number of total arm entries was recorded as a measure of spontaneous locomotor activity.

Forced swim test (FST)

Mice (n=8) were individually placed in a beaker (16 cm in diameter) filled with 20 cm water maintained at 24-25°C for 6 min. Great agitation is usually observed during the first 2 min; immobility generally occurs only during the last 4 min and was recorded during this period. The immobility time was defined as when the mouse ceased trying to swim and performed only movements required to keep its head above water.

Drug and animal treatments

The drugs used in the study included nicotine hydrogen tartrate salt (Sigma, St. Louis, CA, USA), cit-
alopram HBR (Daroupakhsh, Tehran, Iran), and cicitoline sodium (Minoo, Tehran, Iran). All drugs were dissolved in 0.9% saline except for nicotine, which was dissolved in 0.9% saline with the pH adjusted to 7.2 ± 0.1 with NaOH (0.1 N). Nicotine (0.25, 0.5, and 1 mg/kg) was administered subcutaneously (s.c.) at a volume of 10 ml/kg. Citalopram (1.25, 2.5, 5, and 10 mg/kg) and cicitoline (6.25, 12, 25, and 50 mg/kg) were administered intraperitoneally (i.p.) at a volume of 10 ml/kg, 30 min after drug administration. The drug doses were selected based on a pilot study and previous studies (Zarrindast et al., 2012; Nejati et al., 2020). Mice were then subjected to the EPM and then the FST assays. The time interval between the two tests was ten minutes.

Experiment 1

Four groups of mice received saline (10 ml/kg) or nicotine (0.25, 0.5, and 1 mg/kg). Experimental tests were performed 30 min after drug administration. In the experiments, OAT, OAE, locomotor activity, and immobility time were measured as described previously.

Experiment 2

Five groups of mice received saline (10 ml/kg) and different doses of citalopram (1.25, 2.5, 5, and 10 mg/kg) 30 min before tests. The other five groups received nicotine (0.25 mg/kg) and different doses of citalopram (1.25, 2.5, 5, and 10 mg/kg) 30 and 15 min before tests, respectively.

Experiment 3

Five groups of mice received saline (10 ml/kg) and different doses of cicitoline (6.25, 12, 25, and 50 mg/kg) 30 min before tests. The other five groups received nicotine (0.25 mg/kg) and different doses of cicitoline (6.25, 12, 25, and 50 mg/kg) 30 and 15 min before tests, respectively.

Statistical analysis

The Kolmogorov-Smirnov goodness-of-fit test was used to evaluate the distribution normality and variance homogeneity of data. Data were expressed as mean ± SEM. For data analyses, we used one-way and two-way analyses of variance (ANOVA). Following a significant F-value, post hoc analysis was carried out to evaluate specific group comparisons. Differences with P<0.05 between experimental groups were considered statistically significant.

RESULTS

The effect of nicotine on anxiety-like and depression-like responses

Fig. 1 shows the effect of nicotine on the EPM and FST. One-way ANOVA and post hoc analysis revealed that nicotine at a dose of 1 mg/kg decreased OAT (F(3,28)=3.892, P<0.05; Fig. 1A) and OAE (F(3,28)=2.165, P<0.05; Fig. 1B) while increasing immobility time (F(3,28)=3.093, P<0.05; Fig. 1C). Moreover, the injection of different doses of nicotine had no significant effect on locomotor activity (F(3,28)=0.583, P>0.05; Fig. 1D), indicating anxiogenic-like and depressive-like responses.

The effect of nicotine on citalopram response in the EPM and FST

Fig. 2 shows the effects of citalopram, in the presence or absence of nicotine, on anxiety- and depression-related phenotypes in the EPM and FST, respectively. Two-way ANOVA revealed a significant interaction between the effects of nicotine (Factor A) and those induced by citalopram (Factor B) on OAT (Factor A: F(1,70)=27.835, P<0.001; Factor B: F(4,70)=8.833, P<0.001; Factor (A × B): F(4,70)=2.618, P<0.05; Fig. 2A) and immobility time (Factor A: F(1,70)=2.028, P>0.05; Factor B: F(4,70)=18.106, P<0.001; Factor (A × B): F(4,70)=2.820, P<0.05; Fig. 2D) but not on OAE (Factor A: F(1,70)=0.168, P>0.05; Factor B: F(4,70)=1.622, P>0.05; Factor (A × B): F(4,70)=0.017, P>0.05; Fig. 2B) and locomotor activity (Factor A: F(1,70)=1.011, P>0.05; Factor B: F(4,70)=0.906, P>0.05; Factor (A × B): F(4,70)=0.217, P>0.05; Fig. 2C). In addition, based on the post hoc analysis, citalopram (10 mg/kg) increased OAT (F(4,35)=3.205, P<0.05) but decreased immobility time (F(4,35)=4.380, P<0.01) and had no significant effect on OAE (F(4,35)=0.964, P>0.05) or locomotor activity (F(4,35)=0.549, P>0.05), indicating that citalopram elicited anxiolytic-like and antidepressant-like responses (Fig. 2, left panels). Moreover, in the presence of a subthreshold dose of nicotine (0.25 mg/kg), citalopram (10 mg/kg) exerted a significant effect on OAT (F(4,35)=6.745, P<0.01) and immobility time (F(4,35)=18.238, P<0.001) but not OAE (F(4,35)=0.734, P>0.05) or locomotor activity (F(4,35)=0.387, P>0.05) (Fig. 2, right panels). Consequently, the subthreshold dose of nicotine potentiated citalopram’s effects on OAT and immobility time, which revealed anxiolytic-like and antidepressant-like responses.
The effect of nicotine on antidepressant

**Fig. 1.** The effects of various doses of nicotine (0.25, 0.5, and 1 mg/kg) on (A) OAT, (B) OAE, and (C) locomotor activity in the EPM, as well as (D) immobility time in the FST. Data are presented as mean ± S.E.M (n=8). *P<0.05 and **P<0.01 compared to the saline group.

**Fig. 2.** The effects of nicotine administration alone and co-administration of nicotine with citalopram on (A) OAT, (B) OAE, and (C) locomotor activity in the EPM as well as (D) immobility time in the FST. Data are expressed as mean ± S.E.M (n=8). *P<0.05, **P<0.01 and ***P<0.001 compared to the saline group. +P<0.05 and ++P<0.01 compared to the saline/citalopram group.
The effect of nicotine on citicoline-induced response in the EPM and FST

The effects of citicoline alone or in combination with nicotine on anxiety- and depression-related behaviors are shown in Fig. 3. Two-way ANOVA revealed a clear interaction between nicotine (Factor A) and citicoline (Factor B). This interaction was evident for OAT (Factor A: $F_{(1,70)}=23.719$, $P<0.001$; Factor B: $F_{(4,70)}=6.739$, $P<0.01$; Factor $(A \times B)$: $F_{(4,70)}=3.081$, $P<0.05$; Fig. 3A) and immobility time (Factor A: $F_{(1,70)}=1.227$, $P>0.05$; Factor B: $F_{(4,70)}=19.240$, $P<0.001$; Factor $(A \times B)$: $F_{(4,70)}=3.271$, $P<0.05$; Fig. 3D) but not for OAE (Factor A: $F_{(1,70)}=0.065$, $P>0.05$; Factor B: $F_{(4,70)}=5.923$, $P<0.001$; Factor $(A \times B)$: $F_{(4,70)}=0.173$, $P>0.05$; Fig. 3B) and locomotor activity (Factor A: $F_{(1,70)}=0.536$, $P>0.05$; Factor B: $F_{(4,70)}=2.307$, $P>0.05$; Factor $(A \times B)$: $F_{(4,70)}=0.252$, $P>0.05$; Fig. 3C). Further analysis showed that citicoline (50 mg/kg) significantly increased OAE ($F_{(4,35)}=2.962$, $P<0.05$) but decreased immobility time ($F_{(4,35)}=3.521$, $P<0.05$) and did not change OAT ($F_{(4,35)}=1.282$, $P>0.05$) or locomotor activity ($F_{(4,35)}=0.888$, $P>0.05$) (Fig. 3, left panels). The above result suggested that anxiolytic-like and antidepressant-like effects were induced by citicoline. Furthermore, co-administration of a subthreshold dose of nicotine (0.25 mg/kg) with citicoline (50 mg/kg) resulted in a significant effect on OAT ($F_{(4,35)}=7.732$, $P<0.001$), OAE ($F_{(4,35)}=3.101$, $P<0.05$), and immobility time ($F_{(4,35)}=18.735$, $P<0.001$) but not locomotor activity ($F_{(4,35)}=1.514$, $P>0.05$) (Fig. 3, right panels). These results show that the subthreshold dose of nicotine potentiated citicoline’s anxiolytic-like and antidepressant-like effects.

**DISCUSSION**

Nicotine has been revealed to affect anxiety and depression in both human and animal studies (Picciotto et al., 2002). Our present results showed that s.c. injection of nicotine (1 mg/kg) reduced OAT and OAE but increased immobility time and had no effect on locomotor activity. This translates to anxiogenic- and depressive-like responses being elicited by nicotine. Several investigations have indicated that nicotine may induce anxiolytic (Brioni et al., 1994), anxiogenic (Ouagazzal et al., 1999a; Cheeta et al., 2001; Olausson et al., 2001), or neutral effects (Balfour et al., 1986; Olausson et al., 1999; Ouagazzal et al., 1999a) on anxiety-like behaviors, which depend on the species, strain, doses, route of administration, experimental model, and number of trials used (Brioni et al., 1993). Some studies have shown that nicotine elicited anxiogenic-like effects at a high dose (1 mg/kg), while...
lower doses of the drug (0.01 and 0.1 mg/kg) induced anxiolytic-like responses (Ouagazzal et al., 1999b). The pharmacological effects of nicotine are mediated through the stimulation of nAChRs, promoting the release of ACh, 5-HT, NE, dopamine, glutamate, and GABA, the neurotransmitters involved in anxiety (Piri et al., 2012); hence, we proposed that the anxiogenic-like effect induced by nicotine may be due to the activation of nAChRs, which in turn modulate the release of many neurotransmitters. Furthermore, Nguyen et al. (2021) showed that the anxiogenic effect of nicotine is mediated by the inhibition of dopaminergic neurons projecting to the basolateral amygdala and that this effect involved β2-containing postsynaptic nAChRs in the ventral tegmental area (VTA). Morel et al. (2018) demonstrated that nicotine potentiated a stress-induced depressive response through β2 and α7 nAChRs in the VTA. Similarly, Fernandez et al. (2018) suggested that the cholinergic projection from the laterodorsal tegumentum to the VTA is activated by stress and promotes depression-like behaviors. All of these reports highlight the crucial role of dopaminergic signaling in anxiety and depressive behaviors, as shown in many other studies (Chaudhury et al., 2013; Morel et al., 2022).

Also, our findings indicated that injection of nicotine alone induced depressive-like effects by increasing immobility time in the FST. However, co-injection of nicotine and citalopram, as well as nicotine and citicoline, alleviated depression. In mice, nAChRs contribute to the modulation of depression-like responses (Mineur et al., 2018). Furthermore, rodent studies demonstrate that targeting particular nAChR subtypes might provide a therapeutic strategy for treating depression (Mineur et al., 2016). Though no agents acting principally at nAChRs are presently in use for the treatment of depression, it should be noted that numerous current antidepressants, including SSRIs, tricyclics, and bupropion, act as α4β2 nAChR antagonists in cell-based assays (Slemmer et al., 2000; Shytle et al., 2002; Dulawa and Janowsky, 2019). Moreover, nicotine raises serotonergic and noradrenergic neuronal activity and facilitates the release of 5-HT and NE. Previous findings showed that nicotine enhanced the effects of both 5-HT and NE reuptake inhibitors, which suggests an antidepressant action for nicotine (Andreasen and Redrobe, 2009).

Moreover, the results indicated that i.p. injection of citalopram alone or in combination with nicotine enhanced OAT without any significant effect on OAE and locomotor activity but reduced immobility time; citalopram alone or in combination with nicotine-induced anxiolytic-like and antidepressant-like effects. In agreement with our results, several investigations have reported favorable results regarding the use of citalopram in the treatment of depressive and anxiety disorders (Keller, 2000; Baumgartner et al., 2002; Wagner et al., 2004a; 2004b). Citalopram has many potential advantages over other SSRIs, for example, fewer complaints of side effects, mainly initial jitteriness, emotional blunting, and gastrointestinal upset (Keller, 2000; Varia and Rauscher, 2002; Schirman et al., 2010). It also has the highest 5-HT to NE ratio of all SSRIs and a partial weak interaction with the cytochrome-P450 system (Hemeryck and Belpaire, 2002; Lucki and O’Leary, 2004; Schirman et al., 2010).

Citalopram interacts with other compounds, such as nicotine, in the modulation of anxiety and depression (Andreasen and Redrobe, 2009). Moreover, studies have indicated that nicotine administration can increase transcription of 5-HT receptors (Ölausson et al., 1999; Kenny et al., 2001; Picciotto et al., 2002). Specifically, nicotine stimulates the release of 5-HT and NE in many forebrain areas (Mitchell, 1993; Kenny et al., 2000; Ma et al., 2005). Nicotine-induced facilitation of 5-HT and NE release may increase or prolong the effects of drugs that inhibit 5-HT and NE transporters. For example, Popik and colleagues (2003) revealed that nicotine and nAChR antagonists potentiate the antidepressant-like effects of citalopram and imipramine. Interestingly, SSRIs such as citalopram interact with nAChRs and inhibit neuronal nAChRs (Arias et al., 2010a; 2010b). Based on animal studies, citalopram modulates nAChR activity so that non-selective (e.g., nicotine) and α7-selective (e.g., PNU-282987) agonists increase the function of this antidepressant (Popik et al., 2003; Andreasen et al., 2011; Arias et al., 2019).

These reports are in agreement with the hyper-cholinergic hypothesis of depression, where cholinergic activity may become exacerbated relative to the noradrenergic system, and a depressive state may develop; therefore, antidepressant-induced nAChR inhibition could contribute to their mechanisms of function (García-Colunga et al., 2016; Arias et al., 2019). Thus, we proposed an interaction between nicotine and citalopram in which the co-administration of a subthreshold dose of nicotine potentiated citalopram’s effects on OAT and immobility time, indicating anxiolytic-like and antidepressant-like effects.

Our data also showed that administering citicoline alone or in combination with nicotine elicited anxiolytic-like and antidepressant-like responses. Citicoline is a dietary supplement or drug that may have neuroprotective and cognition-improving effects (Brown and Gabrielson, 2012). It is used to treat neurodegenerative diseases such as bipolar disorder, dementia, stroke, traumatic brain injury, and...
addictive disorders. The neuroprotective properties of citicoline may be associated with neuronal membrane phospholipid synthesis and enhancement of several neurotransmitter levels, such as ACh, 5-HT, NE, and dopamine, as well as the improvement of brain tissue markers of oxidative stress (Wignall and Brown, 2014; Roohi-Azizi et al., 2018; Secades and Gareri, 2022). The pharmacological effects of citicoline were found to occur via restoring the insertion of membrane enzymes and enhancing their activity (Secades and Gareri, 2022). Citicoline is distributed throughout the body, crosses the blood-brain barrier, and reaches the CNS. Then, it incorporates into the membrane and microsomal phospholipid fraction (Secades and Lorenzo, 2006; Cho and Kim, 2009). Hence, it prevents neuronal phospholipid membrane breakdown and maintains the neuronal membrane after neuronal damage (Cho and Kim, 2009). Interestingly, citicoline exhibits a modulatory role in anxiety and depression behaviors. In agreement with our results, several studies reported that citicoline reduced anxiety-like responses in the open-field test and EPM (Abdolmaleki et al., 2016; Nejati et al., 2020). Citicoline also displayed an antidepressant effect as an adjuvant drug in unipolar and bipolar depression (Roohi-Azizi et al., 2017). As mentioned above, both nicotine and citicoline increased the level of ACh, 5-HT, NE, and dopamine (Piri et al., 2012; Wignall and Brown, 2014; Roohi-Azizi et al., 2018), so we proposed that cross-talk between nicotine and citicoline affected the modulation of anxiety and depression, such that a subthreshold dose of nicotine potentiated the anxiolytic-like and antidepressant-like properties of citicoline.

CONCLUSION

Based on these results, it can be concluded that there are interactions between nicotine and citalopram or citicoline in the production of anxiolytic and antidepressant effects in mice. Nonetheless, more studies are required to clarify the exact mechanisms of action between nicotine, citalopram, and citicoline in the modulation of anxiety and depression.

REFERENCES


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