Forum Minireview

Functional Proteins Involved in Regulation of Intracellular Ca^{2+} for Drug Development: Chronic Nicotine Treatment Upregulates L-Type High Voltage-Gated Calcium Channels

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Abstract. Neurochemical mechanisms underlying drug dependence and withdrawal syndrome remain unclear. In this review, we discuss how chronic nicotine exposure to neurons affects expression of diazepam binding inhibitor (DBI), an endogenous anxiogenic neuropeptide supposed to be a common substance participating drug dependence, and function of L-type high voltage-gated Ca^{2+} channels (HVCCs). We also discuss the functional interaction between DBI and L-type HVCCs in nicotine dependence. Both DBI levels and [$^{45}\text{Ca}^{2+}$] influx significantly increased in the brain from mice treated with nicotine for long term, which was further enhanced after abrupt cessation of nicotine and was abolished by nicotinic acetylcholine receptor (nAChR) antagonists. Similar responses of DBI expression and L-type HVCC function were observed in cerebral cortical neurons after sustained exposure to nicotine. In addition, increased DBI expression was inhibited by antagonists of nAChR and L-type HVCCs. Sustained exposure of neurons to nicotine significantly enhanced expression of α_1 and α_2/δ_1 subunits for L-type HVCCs and caused an increase in the B_{max} value of [^3H]verapamil binding to the particulate fractions. Therefore, it is concluded that the alterations in DBI expression is mediated via increased influx of Ca^{2+} through upregulated L-type HVCCs and these neurochemical changes have a close relationship with development of nicotine dependence and/or its withdrawal syndrome.

Keywords: anxiety, diazepam binding inhibitor, nicotine dependence, L-type high voltage-gated Ca^{2+} channel, withdrawal syndrome

Although a number of studies have investigated neurochemical mechanisms of drug dependence and withdrawal syndrome, the data are controversial and exact mechanisms have not yet established until now (1, 2). Withdrawal syndrome observed in patients with physical dependence consists of neurological and psychological signs and symptoms and parasympathetic hyperactivity. Among these signs and symptoms, anxiety is a common symptom in physical dependence and withdrawal syndrome in spite of the difference in drugs of abuse inducing physical dependence. Therefore, mechanisms for the development of physical

dependence and/or its withdrawal syndrome may be, in part, mediated via common alterations in neuronal functions. Elucidation of these alterations may provide clues to clarify mechanisms of the development of physical dependence and withdrawal syndrome.

In the present review, we discuss a possible role of diazepam binding inhibitor (DBI) supposed to be a common participant in neurochemical alterations in the drug dependence by nicotine as well as ethanol and morphine (3). We will also discuss the relationship between DBI expression and L-type high voltage-gated calcium channels (HVCCs) in nicotine dependence.

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Endogenous anxiogenic neuropeptide

DBI is a neuropeptide consisting of 86 amino acids with a molecular weight of about 10 kDa and is co-localized with GABA in synaptic vesicles in GABAergic neurons. DBI is distributed in both the central nervous system and peripheral organs (4, 5) and serves as an inverse agonist for benzodiazepine receptors. In deed, the evidence that DBI injected into the rat lateral ventricle induces proconflict responses, which reversed the anticonflict effect of diazepam (6, 7), indicates its role to produce anxiety. Similarly, in a behavioral experiment using a communication box, an increase in DBI expression in brain from mice treated with conditioned emotional stimuli, but not physical stimuli, also suggests that DBI in brain may be a marker to measure anxiety in animals (8). Cerebral DBI expression in brain may therefore participate in anxiety in patients with drug dependence and its withdrawal syndrome.

Nicotine dependence and anxiety

Nicotine is a major component in tobacco and plays a principal role in the psychological and neurological changes induced by smoking. Activation of nicotinic acetylcholine receptors (nAChR) in brain releases various types of neurotransmitters, which in turn causes neuropsychological changes (i.e., alertness and decreased anxiety) (9).

Treatment of rodents with relatively high concentrations of nicotine causes an anxiogenic effect in social interaction tests, whereas that with low doses of nicotine is anxiolytic (10). Moreover, repeated administration of nicotine induces physical dependence and its cessation produces anxiety, depression, sleep disturbance, increased tension, and increased appetite as withdrawal syndrome. In behavioral studies using as an elevated plus maze, α_4 nAChR precipitates anxiogenic disorders (11). Behavioral changes like self-administration of nicotine are not found in mutant mice lacking of β_2 nAChR and mice treated with $\alpha_4\beta_2$ nAChR antagonists (12, 13). These data suggest that involvement of $\alpha_4\beta_2$ nAChRs in the rewarding effects of nicotine. In addition, $\alpha_4\beta_2$ nAChRs are considered to participate in nicotine dependence and appearance of withdrawal syndrome (14–16).

DBI and nicotine-induced anxiety

Anxiety is one of major signs in patients after abrupt withdrawal of smoking, which suggests increase in DBI in the brain. However, a single injection of nicotine (1 mg/kg) to mice produced no alterations in cerebral

DBI mRNA expression up to 3 days after the injection (17). Similarly, exposure of cerebral cortical neurons to nicotine (100 nM) for up to 1–6 h did not change DBI mRNA expression (18). These data indicate that transient contact of neurons to nicotine is not a cause of the increase DBI expression in brain.

Repeated administrations of nicotine to mice for more than 5 days increased both cerebral expression of DBI mRNA and its peptide, and this increase was significantly suppressed by concomitant administration of mecamylamine, an antagonist for nAChR (17). Furthermore, in primary culture of mouse cerebral cortical neurons, exposure to nicotine (0.1 μ M) for more than 24 h increased DBI mRNA expression, and this increase was also completely abolished by hexamethonium (15) (Fig. 1). Taken together with these data, it is indicated that continuous exposure to nicotine is crucial for an increase in DBI expression in brain and that the increase is mediated by continuous activation of nAChR by nicotine. Withdrawal from nicotine further enhanced cerebral DBI mRNA expression and the extent of the expression one day after nicotine withdrawal is higher than that immediately before nicotine withdrawal (19). Such alteration of DBI levels in brain may cause severe anxiety and irritability in patients after abrupt cessation of long-term smoking.

Nicotine and L-type HVCCs

Although mechanisms for the development of drug dependence and withdrawal syndrome have not been

Fig. 1. Effects of hexamethonium and nifedipine on diazepam binding inhibitor (DBI) mRNA expression in cerebral cortical neurons after sustained nicotine exposure. The neurons were cultured with 0.1 μ M nicotine at 37°C for 3 days. ** P <0.01 (Bonferroni's test).

fully clarified, recent investigations suggest that disturbance of Ca^{2+} homeostasis in neurons, especially increased influx of Ca^{2+} , and are partially involved in the drug dependence. In addition, such enhancement of Ca^{2+} entry is also supposed to be due to upregulation of L-type HVCCs in ethanol and morphine dependence. However, in the case of nicotine dependence, few available data have been reported. Therefore, we have examined whether upregulation of L-type HVCCs is induced after sustained exposure to nicotine and whether upregulation of DBI mRNA occurs following increased L-type HVCC function.

Sustained exposure of mouse cerebral cortical neurons to nicotine for 3 days significantly enhanced DBI mRNA expression (20). This increased expression of DBI mRNA was completely abolished by an L-type HVCC inhibitor, nifedipine (Fig. 1), whereas P/Q- or N-type HVCC inhibitors show no effects on nicotine-induced increase in DBI mRNA expression (20). In addition, the steep enhancement of DBI mRNA expression in the neurons noticed after removal of nicotine was also abolished by nifedipine. These results are considered to be relevant to functional modification of L-type HVCCs during nicotine dependence and after withdrawal of nicotine.

Chronic exposure to nicotine significantly increased 30 mM KCl-induced $^{45}\text{Ca}^{2+}$ influx into neurons and the increase was completely abolished by L-type HVCC inhibitors (21). Moreover, long-term exposure to nico-

tine significantly increased the B_{max} value with no changes in the K_d value of ^3H verapamil binding to the particulate fractions prepared from neurons exposed to nicotine, and this increase was significantly suppressed by mecamylamine (19). These data indicate the number of protein molecules composing L-type HVCCs. Western blot analysis on subunits consisting of L-type HVCCs revealed that the immunoreactivities against α_1 and α_2/δ_1 subunits of L-type HVCCs in neuronal membrane obtained from neurons exposed to nicotine were significantly higher than those from the non-treated neurons (21) (Fig. 2). From these data, it is indicated that upregulation of the α_1 subunit of L-type HVCCs induced by long-term exposure of the neurons to low concentration of nicotine causes the increase in DBI expression.

Conclusion

In this review, we demonstrate possible roles of DBI expression and of upregulation of L-type HVCCs and the functional relationship between DBI expression and L-type HVCCs in nicotine dependence. Long-term exposure to nicotine increases both DBI expression and Ca^{2+} entry. The latter is due to upregulation of α_1 and α_2/δ_1 subunits of L-type HVCCs via increment of their protein synthesis. In addition, abolishment of both the increase in DBI levels after long-term exposure to nicotine and transient enhancement of DBI expression

Fig. 2. Changes in expression of L-type high voltage-gated calcium channel (HVCC) subunits in cerebral cortices from mice treated with nicotine for 7 days and in cerebral cortical neurons after sustained exposure to nicotine for 3 days. ** $P < 0.01$, compared with each control value (Bonferroni's test). C: control, N: nicotine-treated.

after withdrawal from nicotine by L-type HVCC inhibitors indicates involvement of L-type HVCCs in DBI expression in nicotine dependence. Such alterations in cerebral DBI expression, especially after nicotine withdrawal, may be also an explanation for the remarkable anxiety observed in patients dependent on nicotine after its withdrawal. Therefore, L-type HVCCs inhibitors are considered to be a candidate to treat nicotine dependence and withdrawal syndrome.

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