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Synaptic transmission in the nucleus tractus solitarius and modes of action of central antitussives

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Summary

It has been presumed that central antitussives exert their action by inhibiting the so-called 'cough center' including the region of nucleus tractus solitarius (NTS) in the brainstem. This review firstly describes synaptic transmission in the second-order NTS neurons that receive peripheral afferents from a variety of visceral receptors. Next, we summarize modulation of the NTS synaptic transmission by several bioactive substances. Finally, the possible modes of action of representative central antitussives, codeine and dextromethorphan, on the excitatory transmission in second-order NTS neurons are proposed.

Keywords: nucleus tractus solitarius, second-order neuron, synaptic transmission, neurotransmitter, neuromodulator, codeine, dextromethorphan

1. Introduction

The central nervous system (CNS) controls the internal environment of the body by generating proper autonomic outputs, which are optimized according to the modality and strength of peripheral inputs from a variety of visceral receptors. Such peripheral afferents converge through the tractus solitarius (TS) on the second-order neurons that compose the local circuit in the nucleus tractus solitarius (NTS) of the brainstem[1]. In addition, the second-order neurons receive excitatory and inhibitory inputs that are generated by interconnections between intrinsic neurons and are thought to modulate in the NTS network activity are able to influence reception and/or integration of the peripheral inputs and to modify the autonomic responses.

Since the second-order neurons in the caudal part of NTS are the first cell receiving cardiopulmonary afferents, this region has been recognized to regulate cardiorespiratory responses via central neuron networks (Fig. 1). Especially, the caudal NTS and its neighboring ventrolateral region are considered as 'cough center' constituting the cough reflex arc.

Cough is a representative respiratory defensive reflex. The vagal afferent signals from the airway rapidly adapting receptors (irritant receptors) and C-fiber receptors are conducted to the second-order

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NTS neurons [4, 5]. The integrated peripheral information activates the central cough/respiratory network to produce a cough pattern. Finally the generated cough motor drive is conducted to the respiratory muscles through efferent motor nerves [6-9].

2. Synaptic transmission in second-order neurons of the nucleus tractus solitarius

Two modes of transmitter release from axon terminals to the second-order neurons are postulated in the NTS; one is spontaneous release and the other is synchronous release. The former represents a spontaneous, stochastic exocytosis from primed vesicles. The latter represents action potential-induced synchronous vesicle exocytosis. Action potential allows the Ca^{2+} entry into the terminal via activation of voltage-dependent Ca^{2+} channels and stimulates a core fusion cascade. Emerging evidence suggests that spontaneous release may arise from a vesicle pool that is regulated separately from that for synchronous release [10].

The results of immunocytochemical, electrophysiological and neuropharmacological studies are in agreement, indicating that glutamate is the main excitatory transmitter in both peripheral and intrinsic excitatory inputs and GABA mediates intrinsic inhibitory inputs in the second-order NTS neurons (Table 1).

2-1. Excitatory inputs

2-1-1. Glutamate

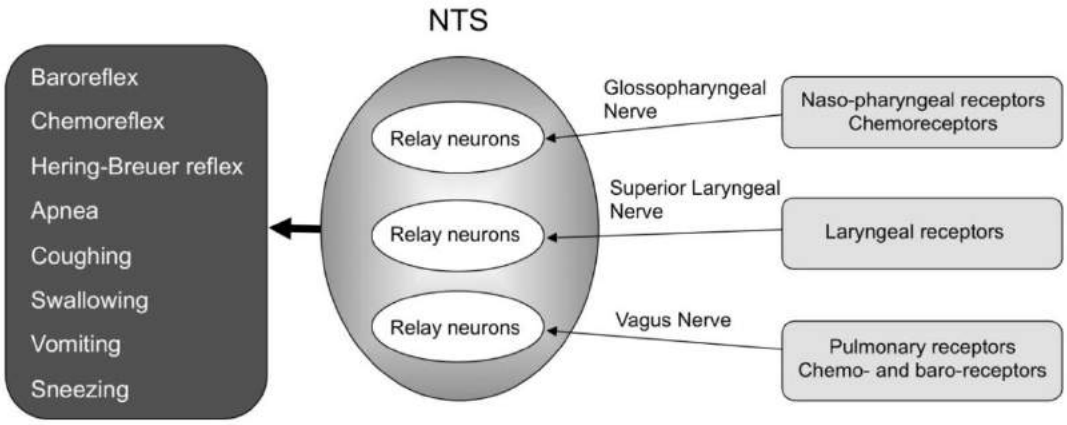

Fig. 1. Cardiorespiratory reflexes through the NTS. See text for details and further information.

Table 1. Neuro-transmitters and -modulators and related receptors in second-order NTS neurons

	Transmitters	Receptors at postsynaptic membrane	Receptors at presynaptic terminal	Modulators	Receptors at postsynaptic membrane	Receptors at Presynaptic terminal
Peripheral Afferent Input	Glutamate	AMPA (+) NMDA (+)	NMDA (-) Group II, III mGluR (-)	Substance P GABA Endocannabinoid 5-HT Noradrenaline Dopamine L-Dopa Adenosine Endomorphine PGE2 PDGF-BB	 5-HT2C (+) PDGF-β (+)	NK-1 (-) GABA-B (-) TRPV1 (-), CB1 (-) 5-HT2 (-) α1 (-) D2 (-) OA1 (via D2) (-) A1 (-) μ (-) EP3 (-)
	Substance P Acetylcholine	NK-1 (+) nicotine (+)	NK-1 (-)			
Intrinsic Excitatory Input	Glutamate	AMPA (+) NMDA (+)	Group I mGluR (+) Group II, III mGluR (-) NMDA (+)	Substance P GABA Endocannabinoid 5-HT Noradrenaline Dopamine L-Dopa ATP Endomorphine PGE2		NK-1 (-) GABA-B (-) TRPV1 (+) 5-HT3 (+), 5-HT1B (-) α1 (-) D2 (-) OA1 (via D2) (-) P2X (+) μ (-) ? (-) or (+)
Intrinsic Inhibitory Input	GABA Glycine	GABA-A (-) Glycine (-)	GABA-A (+) GABA-B (-)	Glutamate Noradrenaline		Group II, III mGluR2 (-) α1 (+)

?: unknown, (+): excitatory effect, (-): inhibitory effect

It is generally accepted that ionotropic glutamate receptors function in the fast-acting neurotransmission, while metabotoropic glutamate receptors (mGluRs) coupled with G-protein modify it [11]. Ionotropic glutamate receptors are roughly divided into two subtypes, N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors including α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA) receptors and kinate receptors. Following evidence suggests that glutamate primarily mediates the excitatory synaptic transmission from both peripheral afferent fibers and intrinsic sources to the second-order NTS neurons [12, 13]. AMPA induces an inward current in the postsynaptic membrane. An antagonist of AMPA receptors markedly blocks both TS-evoked and spontaneous excitatory postsynaptic currents (EPSCs). When the membrane is depolarized to remove Mg^{2+} block from NMDA receptors, NMDA induces an inward current. Therefore, both postsynaptic AMPA and NMDA receptors contribute to the excitatory transmission in the second-order NTS neurons and the former plays a pivotal role. Furthermore, the electron microscopic labeling has confirmed that NMDA receptors are localized at the presynaptic site in the NTS. Activation of presynaptic NMDA receptors exerts an inhibitory effect on TS-evoked release of glutamate but a facilitatory effect on spontaneous release of glutamate [14]. The mGluRs are suggested to be involved in the synaptic transmission in the NTS. Function of Group I mGluRs is inconsistent between animal species. While activation of Group I mGluRs fails to alter the glutamatergic transmission in the rat NTS neurons [15], the mGluR1 mechanism facilitates the release of glutamate from intrinsic terminals in the guinea pig NTS neurons through the presumably activation of Gq protein and increase in Ca^{2+} concentration [16]. Group II and III mGluRs play

an inhibitory regulation of glutamate release from intrinsic interneurons and peripheral afferent terminals in the rat [15] and guinea pig [16].

2-1-2. Substance P

The intermediate and caudal NTS are enriched with substance P-containing axon terminals [17], which emanate from capsaicin-sensitive vagal afferent C fibers and terminals with cell bodies originating within the NTS and higher brain centers. Depletion of substance P in the vagus nerve blocks rapid shallow breathing and apnea induced by pulmonary congestion [18]. Local application of substance P [19] or a selective neurokinin 1 receptor agonist [20] depolarizes the NTS neurons, suggesting the postsynaptic excitatory action of substance P. Moreover, it has been demonstrated that substance P decreases both TS-evoked EPSCs with an increase in the paired-pulse ratio, and the frequency of spontaneous and miniature EPSCs in the second-order NTS neurons [21]. This proposes a new role of substance P, which is a presynaptic depression of glutamatergic transmission between the broncho-pulmonary afferents and second-order neurons.

2-1-3. Acetylcholine

Acetylcholine (ACh) is broadly distributed throughout the CNS and contributes to central autonomic regulation. Cholinergic systems are identified by the presence of choline acetyltransferase, ACh esterase and ACh in the NTS [22]. Microinjection of ACh or nicotine into the NTS elicits hypotension and bradycardia similarly to stimulation of the arterial baroreceptors. Nicotinic receptors are also involved in tachypnea induced by the peripheral chemoreceptor activation. ACh modulates the activity of sympathetic nerve and phrenic nerve via nicotinic receptors. Iontophoresis of ACh causes excitation of NTS neurons. Nicotine induces an inward current, and this effect is blocked by hexamethonium. Together, ACh mediates, in part, the signal transmission from sensory afferents to the NTS neurons via postsynaptic nicotinic receptors [23].

2-2. Inhibitory inputs

2-2-1. GABA

In addition to the excitatory synaptic inputs, the NTS neurons receive inhibitory inputs from intrinsic sources. A balance of these two mechanisms controls the net activity of neurons. Spontaneous inhibitory postsynaptic currents (IPSCs) in the second-order NTS neurons are reversed at the membrane potential similar to an equilibrium potential of Cl^- and markedly blocked by $GABA_A$ antagonists [2]. These results indicate that GABA mediates the intrinsic inhibitory transmission in the second-order NTS neurons by activating the postsynaptic $GABA_A$ receptors. Moreover, GABA inhibits glutamate release from the afferent terminals via $GABA_B$ receptors [24], and conversely, GABA release is modulated by glutamate through mGluRs [25]. This heterosynaptic modulation implies that the glutamatergic and GABAergic terminals are in close proximity. In mechanically isolated NTS neurons, the spontaneous EPSC frequency is increased by $GABA_A$ receptor agonists, but decreased by $GABA_B$ receptor agonists. The antagonists of $GABA_A$ and $GABA_B$ receptors exert opposite effects. Either Na^+-K^+-Cl^- co-transporter type 1 antagonist or Na^+/Ca^{2+} channel inhibitor blocks facilitation of the $GABA_A$ receptor-mediated release of glutamate, indicating $GABA_A$-mediated presynaptic depolarization [26]. Thus, tonically released GABA activates $GABA_A$ and $GABA_B$ receptors to modulate the release of glutamate. These findings provide cellular mechanisms of heterosynaptic GABA-glutamate integration of peripheral visceral afferent signals in the NTS.

2-2-2. Glycine

Glycine is one of the two major inhibitory neurotransmitters in the CNS. Mixed GABA-glycine axon terminals are located at the lateral part of NTS

within subnuclei surrounding the TS. Pure glycine axon terminals are rare in the lateral part and hardly detected in the medial part. Electrophysiological experiments have demonstrated existence of a dual inhibition involving the co-release of GABA and glycine, and confirmed predominance of GABA inhibition in the NTS. Glycine receptors are expressed postsynaptically in the NTS together with $GABA_A$ receptors. Glycine induces inhibitory responses of the NTS neurons [27]. Furthermore, glycine is mostly associated with GABA within axon terminals, suggesting the possibility of a dynamic regulation of GABA/glycine release at the presynaptic level [28].

3. Modulation of the synaptic transmission in second-order neurons

The NTS is extremely rich in receptors for many endogenous bioactive substances, and their activation strongly influences the signal processing in this region (Table 1).

3-1. Endocannabinoidergic mechanisms

Endocannabinoids are derived from lipid metabolites. They closely resemble the chemical structure of vanilloid agonists and can activate transient receptor potential vanilloid 1 (TRPV1), a nonselective cation channel, which is sensitive to high temperature (> 43°C), protons and chemical substances. The afferent terminal fields within the NTS appear strongly immunoreactive for TRPV1 channels. Second-order NTS neurons receive the TS afferent inputs that either express TRPV1 channels ($TRPV1^+$) or not ($TRPV1^-$) [29]. TS-evoked synchronous release of glutamate is indistinguishable between $TRPV1^+$ and $TRPV1^-$ afferent terminals, while $TRPV1^+$ afferents display 10-fold higher rate of spontaneous release of glutamate than $TRPV1^-$ afferents. Activation of TRPV1 channels by capsaicin inhibits TS-evoked EPSCs but increases spontaneous EPSCs [30]. Most TS afferents are $TRPV1^+$ and the rate of spontaneous transmitter release depends closely on temperature in the physiological range. This thermally driven glutamate release persists when Ca^{2+} entry through voltage-dependent Ca^{2+} channels is blocked. TRPV1 channels play a key role in neonatal respiratory regulation with small temperature shifts within the NTS.

On the other hand, endocannabinoids activate cannabinoid type 1 (CB_1) receptors and broadly inhibit cardiovascular and gastrointestinal functions [31]. Activation of CB_1 receptors decreases TS-evoked synchronous release of glutamate in the second-order NTS neurons, while it has no effect on the spontaneous release [32]. CB_1 receptors and TRPV1 channels differently regulate glutamate release despite co-existence in the same terminations. This two-pool arrangement allows independent and opposite modulation of glutamate release by single lipid metabolites. Therefore, the synaptic transmission from TS afferents with $TRPV1^+$ or $TRPV1^-$ serves as a unique model to assess CB_1 receptors/TRPV1 channels interactions in the release of glutamate. Additionally, activation of CB_1 receptors expresses long-term depression thorough stimulating the presynaptic NMDA receptors in the NTS.

3-2. Serotonergic mechanisms

The NTS neurons are innervated by serotonergic (5-HTergic) terminals from the raphe nuclei [33] and vagal afferents [34]. Immunohistochemical and mRNA analyses have revealed the existence of $5\text{-}HT_{1A}$, $5\text{-}HT_2$, $5\text{-}HT_3$, $5\text{-}HT_{5A}$ and $5\text{-}HT_7$ receptors in the NTS. 5-HT is suggested to modify cardiorespiratory functions including related reflexes. Activation of $5\text{-}HT_2$ and $5\text{-}HT_3$ receptors causes apnea and that of $5\text{-}HT_{1A}$ receptors inhibits cough reflex in animal models. Stimulation of $5\text{-}HT_3$ receptors within the NTS results in a rise in blood pressure and inhibition of bradycardia evoked by chemoreceptor activation. The $5\text{-}HT_{2A}$ mechanism facilitates the baroreceptor reflex by functional interaction with NMDA receptors co-expressed in the same neuron. Various effects of 5-HT on the NTS neuron activity or

synaptic transmission have been demonstrated. The spontaneous activity is inhibited by 5-HT_2 and 5-HT_{1D} mechanisms, but enhanced by 5-HT_{1B} and 5-HT_7 mechanisms. 5-HT inhibits TS-evoked EPSCs in the second-order NTS neurons via presynaptic 5-HT_2 receptors while facilitates spontaneous EPSCs via presynaptic 5-HT_3 receptors [35]. Furthermore, 5-HT_{2C} receptors at the postsynaptic membrane are related to facilitation of excitatory transmission from both TS afferent and intrinsic inputs [36].

3-3. Adrenergic mechanisms

Noradrenaline is a neurotransmitter in central autonomic regulation. Peripheral chemoreceptor stimulation activates central noradrenergic structures. Phenylephrine, an α_1 receptor agonist, decreases the amplitude of TS-evoked EPSCs in the caudal NTS neurons and this effect is blocked by prazosin, an α_1 receptor antagonist. Phenylephrine is without effect on the basal current, input resistance and current-voltage relationship. The frequency of miniature IPSCs is increased by phenylephrine, but that of miniature EPSCs is decreased. These results suggest that activation of α_1 receptors reduces excitatory inputs and enhances inhibitory inputs in the second-order NTS neurons via presynaptic mechanisms [37]. It is suggested that noradrenaline plays an inhibitory role in the synaptic transmission in the NTS via α_1 receptors and modulates the autonomic responses to hypoxia.

3-4. Dopaminergic mechanisms

Dopamine (DA) is presented in the chemoreceptor afferent fibers synapsing to the second-order NTS neurons and plays an important step in cardiorespiratory reflexes [1]. D2 receptors and/or mRNA are found in the vagal afferent neurons and NTS, while D1 receptors are not yet. Microinjection of DA into the NTS produces either pressor effect with tachycardia or depressor effect with bradycardia. During hypoxia, occurrence of respiratory depression coincides with a rise in DA concentration in the NTS. This increase in DA content is eliminated by section of the carotid sinus nerve. DA decreases TS-evoked EPSCs together with an increase in the paired-pulse ratio, but had neither effect on the EPSC decay nor input resistance of the membrane [38]. Similar results are obtained by a D2 receptor agonist but not by a D1 receptor agonist. DA decreases the frequency of miniature EPSCs and sulpiride, a D2 receptor antagonist, has an opposite effect. DA also inhibits spontaneous IPSCs through D2 receptors [39]. Immunohistochemical study shows co-localization of D2 receptors with synaptophysin. Together, DA tonically modulates synaptic activity between the afferent sensory fibers and second-order NTS neurons via presynaptic D2 receptors.

3-5. L-dopaergic mechanisms

L-dopa, the metabolic precursor of DA, is suggested to be one of neuromodulators of baroreflex pathways in the NTS, since stimulation of the aortic depressor nerve causes release of L-dopa in parallel with induction of hypotension and bradycardia. Moreover, L-dopa functions to stimulate the caudal ventrolateral medulla (CVLM) that is a depressor site receiving inputs from the NTS [40]. Lesion of the NTS selectively decreases the tissue content of L-dopa in the ipsilateral CVLM. The gene product of ocular albinism 1 (OA1), which is coupled with Gq-protein, possesses L-dopa-binding activity and is expressed in the NTS. OA1 knockdown suppresses the depressor and bradycardiac responses to L-dopa, indicating that OA1 is a functional receptor for L-dopa in the NTS [41]. In the second-order NTS neurons, L-dopa decreases both the frequency of miniature EPSCs and TS-evoked EPSCs together with an increase in the paired-pulse ratio. These effects are blocked by an OA1 antagonist and also by a D2 antagonist. Furthermore, the L-dopa's effects are completely abolished in reserpine-pretreated rats. These results propose a possibility that L-dopa may release DA via OA1 receptors located at the DA axon terminals and the released DA decreases the

glutamate transmission through activation of presynaptic D2 receptors [42].

3-6. Purinergic mechanisms

Purinergic receptors in the caudal NTS are required for normal cardiovascular reflexes [43]. Extracellular purines, such as adenosine and adenosine 5-triphosphate (ATP), are the primary mediators signaling emergency changes in the internal environment in the CNS. Expression of adenosine transporter, A1 receptors and adenosine deaminase in the NTS suggests roles of purinergic signaling in the NTS. The afferent terminal fields in the NTS appear strongly immunoreactive for P2X receptor subtypes and their mRNA. P2X3 receptors are localized at presynaptic terminals containing glutamate [44]. ATP activates presynaptic P2X receptors on the axon terminals of intrinsic excitatory NTS neurons facilitating spontaneous release of glutamate and also presynaptic adenosine A1 receptors, after being hydrolysed to adenosine, reducing evoked release of glutamate from the primary afferent terminals. Thus, ATP modulates excitatory synaptic inputs arising from distinct origins and converging on single postsynaptic neuron in opposite directions through activation of distinct presynaptic purinoceptors [45, 46]. ATP also induces a fast inward current in a subset of acutely isolated NTS neurons, suggesting a postsynaptic mechanism [47].

3-7. Opioidergic mechanisms

Opioid receptors including three subtypes (μ-, δ- and κ-receptors) are present on the NTS dendrites and the vagal afferent terminals [48]. Endomorphine 1 and 2 (EM-1 and EM-2) are endogenous opioidergic peptides with high affinity and selectivity for the μ-receptor in mammals [49]. Immunohistochemical studies have shown that EM-1 staining is found in cell bodies and terminal fields within the NTS, and is closely related to the staining of μ-receptors. EM-2 staining is located at varicose fibers, and perhaps within the TS. EM-1 decreases the amplitude of TS-evoked EPSCs with an increase in the paired-pulse ratio, but does not affect input resistance. EM-1 decreased spontaneous EPSCs and IPSCs in frequency but not in amplitude. These results suggest that EM-1 acts at presynaptic μ-receptors to inhibit the peripheral inputs and the intrinsic inputs from local synaptic circuitry in the NTS [50]. Similarly, activation of μ-receptors by DAMGO inhibits the TS-evoked and spontaneous EPSCs in the NTS neurons [51, 52].

3-8. Prostaglandin E_2-mediated mechanisms

Prostaglandin E_2 (PGE_2) exerts profound effects on various physiological functions such as temperature regulation, circulation and nociception by acting the neural networks. PGE_2 receptors are rich in the medial part of NTS [53]. PGE_2 enhances chemical and mechanical sensitivities of C fibers. PGE_2 potentiates tetrodotoxin-resistant Na^+ currents in capsaicin-sensitive pulmonary sensory neurons and peripheral nerve terminals, while it inhibits voltage-dependent Ca^{2+} currents in the NTS neurons. The effects of PGE_2 on synaptic transmission in second-order NTS neurons are still controversial; either excitatory, inhibitory or no effect. For instance, PGE_2 increases the amplitude of TS-evoked EPSCs in NTS neurons [54] or decreases it via presynaptic EP3 receptors coupled with G-proteins linked to adenylyl cyclase and protein kinase A activity [55, 56]. PGE_2 increases the frequency of miniature [54] and spontaneous EPSCs [56] through a presynaptic mechanism different from EP1-4 receptor mechanisms. On the contrary, there is a report that PGE_2 decreases the frequency and amplitude of spontaneous and miniature EPSCs [55]. Moreover, PGE_2 interacts with TRPV1 channels and 5-HT_3 receptors [57].

3-9. Platelet-derived growth factor-mediated mechanisms

Platelet-derived growth factor (PDGF)-BB is

released in the NTS during hypoxia together with glutamate from chemoreceptor afferents. Hypoxic ventilatory response is inhibited by microinjection of PDGF-BB, but not PDGF-AA, into the dorsocaudal brainstem, which activates the PDGF receptor (PDGFR)-β [58]. A marked reduction of the late decline in hypoxic ventilatory response occurs in mice heterozygous for a mutation in the PDGFR-β. An antagonist of PDGFR-β also reduced the late decline in hypoxic ventilatory response in wild-type littermates. Blockade of NMDA receptors attenuates the reduction of late decline in hypoxic ventilatory response induced by the PDGR-BB/PDGFR-β signal [58, 59]. Exogenous PDGF-BB postsynaptically decreases the TS-evoked EPSCs in the second-order NTS neurons, suggesting that the PDGF-BB/PDGFR-β signal inhibits the glutamatergic transmission [60]. This might participate in controlling the ventilatory response during hypoxia.

4. Modes of action of central antitussives on the second-order neurons

4-1. Cough-gating mechanism

Cough is produced in the cough reflex arc constituted of airway afferent nerves, the central cough network (cough center) and respiratory efferent nerves [3, 7, 9, 61]. Electrical microstimulation of the caudal NTS including the neighboring ventrolateral area induces cough reflex and lesion of the NTS abolishes cough induced by stimulation of afferent nerves [9, 62]. It has been proposed the presence of a cough-gating mechanism in the caudal NTS, which receives afferent inputs and sends a triggering signal for cough generation to the cough/respiratory network [6, 7]. The cough-gating mechanism is able to account for the fact that antitussives do not alter breathing at doses that inhibit cough, suggesting the presence of a neuronal component important for cough that does not participate in breathing pattern generation. Therefore, the caudal NTS is a main region for the cough center and is postulated to be the site of action of central antitussives.

4-2. Codeine

Codeine is the most widely used centrally-acting drug to suppress excessive cough. Microinjection of codeine into the caudal NTS suppresses fictive cough responses in animal models [9]. Intravenous codeine inhibits the depolarization of cough-related NTS neurons in parallel with suppression of fictive cough induced by electrical stimulation of afferent nerves [6, 63, 64]. Codeine binds to μ-, δ- and κ-receptors with different affinities [65] and decreases transmitter release by activating K^+ channels and/or inhibiting N- and P/Q-type Ca^{2+} channels. It has been demonstrated in the second-order NTS neurons of the brainstem slice that codeine (0.3-3.0 mM) inhibits TS-evoked EPSCs by activating μ-receptors- and 4-aminopyridine-sensitive K^+ channels at presynaptic sites [66]. Furthermore, codeine decreases the frequency of spontaneous and miniature EPSCs through activating presynaptic μ- and κ-receptors which are independent on K^+ and Ca^{2+} channels [52]. These results suggest that the antitussive effects of codeine are, at least partly, derived from interruption of the peripheral afferent inputs to the second-order neurons and from dampening of the basal activity of NTS network. The concentration of codeine is calculated at 0.4-1.2 mM based on the total volume of blood (7 ml/100 g), when codeine is administered into guinea pigs at 10-30 mg/kg subcutaneously or orally which was reported to inhibit cough reflex in guinea pig cough model. This is equivalent to the effective concentration of codeine (0.3-3.0 mM) to inhibit the NTS synaptic transmission.

4-3. Dextromethorphan

Dextromethorphan (DEX), a non-opioid dextrorotatory morphinan derivative, is thought to elevate the cough threshold and suppress coughing [67]. DEX inhibits voltage-dependent Na^+ and Ca^{2+} channels. Further, DEX acts as an antagonist of

NMDA receptors [68] or an agonist of σ receptors [69]. The drugs having characteristics of blocking the NMDA receptors [62] and of stimulating the σ receptors possess the antitussive activity. In the second-order NTS neurons, DEX (0.1-1.0 mM) reversibly decreases the TS-evoked EPSC with an increased paired-pulse ratio in a concentration-dependent manner. Neither a σ-1 receptor antagonist nor a σ-1 and -2 receptor ligand, haloperidol, blocks the DEX-induced inhibition of TS-evoked EPSC. DEX decreases the frequency of miniature EPSCs without effect on their amplitude. DEX has no effect on an inward current induced by exogenous AMPA in the NTS neurons. These results suggest that DEX inhibits glutamate release from the presynaptic terminals projecting to the second-order NTS neurons, but this effect of DEX is not mediated by the σ receptors [70]. DEX possesses the antitussive activity when the dose of 30-60 mg/kg is injected intraperitoneally into the guinea pig in vivo, where its concentration in the blood is estimated to be 1.1-2.2 mM. This calculated concentration is comparable with that of DEX (0.1-1.0 mM) that inhibits synaptic transmission in the NTS neurons. However, since the IC_{50} of DEX (0.01-0.1 mM) for the NMDA-induced current and the voltage-dependent Na^+ and Ca^{2+} currents in cultured neurons is much lower, these mechanisms may not be related to the antitussive action of DEX.

5. Conclusion

Although glutamate and GABA play a pivotal role in synaptic transmission in the NTS, afferent information is distributed to a number of destinations after arriving at the first relay neuron in the brainstem, and these circuits may use several neurotransmitters. The caudal NTS constitutes a kernel of the cardiorespiratory reflex pathways. Particularly, the central organization of the cough reflex involves the caudal NTS network. It is important to clarify the modes of action of neurotransmitters and neuromodulators and also the inhibitory mechanism of central antitussives in the NTS network. Neuropharmacological investigation on the NTS synaptic transmission may provide the basis of novel therapeutic approaches for cough reflex.

Conflicts of interest

The authors declare no conflicts of interest, financial or otherwise.

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