*Current Perspective***Multiple Pharmacological Actions of Centrally Acting Antitussives — Do They Target G Protein-Coupled Inwardly Rectifying K⁺ (GIRK) Channels?**Kazuo Takahama^{1,*}¹Department of Environmental and Molecular Health Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

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Abstract. Antitussive drugs have been used for decades and their modes of action are well elucidated. However, recent studies on the mechanism of their antitussive action seem to be opening a new way for discovery or development of novel drugs for intractable brain diseases including psychiatric disorders. Antitussives inhibit the currents caused by activation of G protein-coupled inwardly rectifying K⁺ (GIRK) channels in neurons. In our own studies carried out so far, we found that antitussives possessing an inhibitory action on GIRK channels, similar to the effects of an enriched environment, ameliorate symptoms of intractable brain diseases in various animal models. In this review, the multiple pharmacological actions of the antitussives are described, and their mechanisms are discussed addressing GIRK channels as a possible molecular target.

Keywords: G protein-coupled inwardly rectifying K⁺ (GIRK) channel, centrally acting antitussive, antidepressant-like action, enriched environment, dopamine

1. Introduction

In our study on the mechanisms of the antitussive action of cough suppressant drugs, we found that dextromethorphan, a centrally acting non-narcotic antitussive, inhibits G protein-coupled inwardly rectifying K⁺ (GIRK) channel-activated currents mediated by 5-HT_{1A} receptors in dorsal raphe neurons of rats, although the drug also inhibited the inwardly rectifying K⁺ currents regulated by α_2 -adrenoceptors in rat locus coeruleus neurons (1). Further studies demonstrated that this is also the case for other centrally acting antitussives (2). GIRK channels are involved in the formation and maintenance of resting membrane potentials, K⁺ homeostasis, inhibition of synapses, and determination of firing frequency of action potentials in excitable cells (3). Because GIRK channels are coupled to various G protein-coupled recep-

tors (GPCRs) in the brain (3), cough suppressant drugs that inhibit GIRK channel-activating currents are expected to have various pharmacological effects. This review describes the multiple pharmacological actions of antitussives, addressing GIRK channels as a target for developing new therapeutic drugs for intractable brain diseases.

2. Action of antitussives on GIRK-channel currents

A few studies have suggested that the cough suppressant action of non-narcotic antitussives may be caused by activation of 5-hydroxytryptamine 1A (5-HT_{1A}) receptors in the brain. Our own study using the patch clamp technique revealed that dextromethorphan, a non-narcotic antitussive, fails to produce the current through activation of 5-HT_{1A} receptors in single neurons isolated from the raphe nucleus of guinea pigs, suggesting that the drug itself had little action on 5-HT_{1A} receptors in brain neurons of the raphe nucleus. Unexpectedly, dextromethorphan inhibited the 5-HT-induced currents caused by activation of 5-HT_{1A} receptors (1). This action was confirmed for other non-narcotic antitussives (2). In neurons

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intracellularly perfused with GTP γ S, a 5-HT_{1A}-receptor antagonist did not block the 5-HT_{1A} receptor-mediated inward current. However, dextromethorphan did abolish the current, suggesting that the drug may block the GIRK channel coupled to the 5-HT_{1A} receptor (Fig. 1) (1). This action was also found for other antitussives. Furthermore, an NMR study combined with electrophysiological study using *Xenopus* oocytes suggested that cloperastine, another antitussive, may interact with the channel pore region of the GIRK channel (unpublished observation; this study was done in collaboration with Dr. Shimada and Dr. Oosawa from The University of Tokyo).

I was strongly concerned about the finding that all centrally acting antitussives examined inhibit GIRK channel-activated currents with a relatively high potency, despite the diversity of their chemical structures. Further analysis of the relationship between the antitussive activity and the inhibitory action on GIRK channel-activated current of centrally acting antitussives revealed a significant correlation between the two, suggesting that GIRK channels may be a target of the antitussive action of the drugs. This observation was also supported by verifying the 'piperidino group theory' for the structure-activity relationship of centrally acting antitussives (4), that is, by determining the action of various antitussive drugs with or without a piperidino group on GIRK channel-activated currents (2).

Fig. 1. Effect of dextromethorphan on the 5-HT-induced current irreversibly activated by intracellular GTP γ S. The neurons were intracellularly perfused with an internal solution containing 0.1 mM GTP γ S by using the conventional whole-cell patch recording mode. Current recording was performed in external solution containing 20 mM K⁺ at a V_H of -80 mV. Please note that dextromethorphan (DM) almost completely reversed 5-HT-induced current in the neuron. Cited from Ref. 1.

3. Multiple pharmacological actions of antitussives

3.1. Action on micturition reflex

Based on a bold analogy of both the responses of coughing and micturition, we tested whether centrally acting antitussives depress the micturition reflex in experimental animals. Interestingly, dextromethorphan ameliorated the reduced latency of micturition associated with cerebral infarction caused by occlusion of the mid-cerebral artery in rats (5), but did not ameliorate infarction-induced increase in urinary resistance. In addition to the inhibitory action on GIRK channel-activated current, dextromethorphan inhibits the *N*-methyl-D-aspartate (NMDA)-induced current (I_{NMDA}) in brain neurons. MK-801, a non-competitive NMDA-receptor antagonist, reportedly increases the threshold pressure of the micturition reflex and shows residual urine in the bladder of rats, suggesting an increase in urethral resistance. Here, we considered the following idea: a drug that possesses a GIRK channel-inhibiting action but not a NMDA receptor-blocking action may ameliorate the reduced latency of micturition associated with cerebral infarction without increasing urethral resistance. Therefore, we next determined the action of cloperastine on the micturition reflex in rats with cerebral infarction because the drug has little action on I_{NMDA} and a potent inhibitory action on GIRK channel-activated currents. As expected, cloperastine strongly ameliorated both the symptoms of detrusor overactivity and detrusor sphincter dyssynergia in conscious rats with cerebral infarction (5).

Based on several lines of evidence, it is likely that the ameliorating effect of cloperastine on the disturbed micturition reflex may be caused at least partly by inhibition of GIRK channels in the brain. GIRK channels are distributed in the raphe nuclei, locus ceruleus, periaquiductal gray matter, and prefrontal cortex, all of which are implicated in the micturition reflex (6, 7). The serotonergic neurons arising from the raphe nuclei innervate not only the forebrain but also the dorsal horn, intermediolateral nucleus, and Onuf's nucleus in the lumbosacral spinal cord. These regions are also implicated in the micturition reflex. Serotonergic 5-HT_{1A} and 5-HT_{1B} receptors are localized in the lumbosacral spinal cord, where preganglionic neurons innervating the urinary bladder are located (8). Because electrical stimulation of the raphe nuclei inhibits bladder activity in the cat (9), excitation of the raphe nuclei should inhibit the micturition reflex. Our previous study using single raphe neurons revealed that cloperastine and dextromethorphan induce firing of the neurons inhibited by 5-HT-induced activation of 5-HT_{1A} receptors (2). 5-HT_{1A} receptors are coupled to GIRK channels and are localized in the raphe neurons. In addition, the GIRK1 and GIRK3 subunits of GIRK

channels also exist in raphe neurons. Therefore, antitussive-induced firing of raphe neurons is likely to be caused by inhibition of GIRK channels in the raphe neurons. In this context, it is reasonable to speculate that the ameliorating effects of dextromethorphan and cloperastine on detrusor overactivity following cerebral infarction may result at least in part from an increase in 5-HT release via the inhibition of GIRK channels (5).

Cloperastine also ameliorated the decrease in urinary flow associated with the increase in urinary resistance in conscious rats with cerebral infarction. Although the mechanism is largely unknown, we hypothesized that amelioration of the dysuria might be also at least partly due to the GIRK channel-inhibiting action of cloperastine. Our preliminary study showed that antidepressants, which increase 5-HT level in the brain, ameliorated bladder overactivity in rats with cerebral infarction, but had no effect on the symptoms of dysuria at all. However, only fluoxetine possessing a weak inhibitory action on GIRK channel-activated currents tended to improve the symptoms of dysuria.

Based on the above findings, I proposed a bold working hypothesis that antitussives with GIRK channel-blocking action may produce their pharmacological actions through stabilizing an excitatory state (or abnormal state) in the brain (2), because coughing and urination are both controlled by the brain. For the first step in verifying this hypothesis, we studied whether centrally acting antitussives inhibit or ameliorate various symptoms such as methamphetamine-induced hyper-locomotion, endocrine disruptor (ED)-induced impairment of passive avoidance responses, and others. Surprisingly, the antitussives ameliorated the various symptoms of drug-induced disturbance of brain functions. Notably, many of these symptoms are difficult to treat with preexisting therapeutic drugs. Furthermore, it should be noted that the ameliorative effects were produced at a dose in the range similar to that for the antitussive effect, suggesting that these effects might be produced through the same mechanism. The effects of antitussive drugs are on the following disorders: animal models of attention deficit hyperactivity disorder (ADHD), Parkinson's disease, depression, anxiety, schizophrenia, and brain disturbance caused by ED. Among these, the actions of antitussives on depression and brain disturbance caused by ED are described next.

3.2. Action on brain-disturbance caused by ED

We previously found that prenatal exposure of mice to a very low dose (0.1 μ g/animal per day from gestational day 11th to 17th) of diethylstilbestrol (DES), a prototypical ED, causes impairment of passive avoidance responses (10). Apart from this, I noted Kempermann's

paper (11), which reported that rearing of rodents in an enriched environment increases the number of hippocampal neurons and facilitates learning behaviors. Combining these observations, I thought of the intriguing idea that the brain disrupting actions of DES may be reversed in rodents reared in an enriched environment because the major brain-disrupting actions of DES are learning disability. As expected, the brain-disrupting actions of DES were reversed when the mice were reared in an enriched environment consisting of a plywood cage (100-cm-wide $\times$ 100-cm-long $\times$ 30-cm-high) in which various toys, a house, running wheels, and a tunnel were arranged (12). Interestingly, environmental enrichment ameliorated the symptoms of animal models of various intractable brain diseases such as Alzheimer's disease and Huntington's disease (13). On the other hand, antitussives also improved various symptoms of brain diseases as described in the previous paragraph. Thus, I hypothesized that the antitussives may mimic environmental enrichment. As expected, repeated administration of cloperastine at doses of 10 or 30 mg/kg twice a day ameliorated DES-induced impairment of passive avoidance responses in mice (14). Although the mechanisms of the effect of cloperastine are unknown, environmental enrichment and antitussives such as cloperastine both increase the dopamine level in the nucleus accumbens (NAc) of the brain (15, 16). Recently, increased dopamine levels in the NAc have received attention because such an increase may have therapeutic potential for various intractable brain diseases such as intractable depression (17). A cloperastine-induced increase in the dopamine level in the NAc is likely caused by the inhibitory action on the GIRK channels in the ventral tegmental area (VTA), as described in the next paragraph. However, the mechanism of this increase in an enriched environment is unknown.

3.3. Effects on models of depression

Currently available antidepressants from the first to the fourth generation have been developed based on the monoamine theory for the etiology of depression. The core of the theory is that depression may be caused by lower levels of monoamines such as serotonin, noradrenaline, and dopamine in the brain. Because these monoamines have different psychiatric functions, I expected that drugs such as tipepidine with GIRK channel-inhibiting action might lead to changes in the balance of monoamine levels in the brain, resulting in improvement in the symptoms of depression. GIRK channels are coupled to various GPCRs such as 5-HT_{1A}, adrenaline α_2 , and dopamine D₂ receptors in the brain. Therefore, inhibition of these GIRK channels should excite neurons possessing GPCRs, resulting in an increase in the level

of the corresponding monoamines (Fig. 2). Our own preliminary studies using microdialysis confirmed this phenomenon in the prefrontal cortex and NAc in rats (16). As expected, tipepidine decreased the immobility time in the forced swimming test in rats (18) and mice, suggesting that the drug may have an antidepressant-like effect. When three parameters of immobility, swimming, and climbing in the forced swimming test were considered, the neurobehavioral profile of tipepidine was observed to be quite different from that of preexisting antidepressants (18). Surprisingly, tipepidine also showed an antidepressant-like action in adrenocorticotrophic hormone-treated rats (19, 20) in which the immobility time is not improved by known antidepressants such as imipramine (21, 22). The precise mechanism of the action of tipepidine is unknown, but it is likely to be at least partly by inhibiting GIRK channels coupled to GPCRs (23). We found that caramiphen, another centrally acting antitussive that inhibits GIRK channel-activated currents, also has antidepressant-like action with a very similar neurobehavioral property to that of tipepidine (24), although the chemical structures of both drugs are quite different. Furthermore, pharmacological studies suggested that tipepidine may cause an antidepressant-like effect at least partly through the action of dopamine and

noradrenaline released by inhibiting GIRK channels on dopamine D₁ and adrenaline α_2 receptors (23). In addition, our recent preliminary studies using microinjection showed that tipepidine might cause antidepressant-like action by exciting the dopaminergic neurons projecting from the VTA to the NAc (25). Again, the excitation of dopaminergic neurons in the VTA should be caused by inhibition of GIRK channels because GIRK1 and GIRK2 subunit mRNA are expressed in the VTA. In a preliminary electrophysiological study, we found that tipepidine and other GIRK channel-inhibiting drugs inhibit GIRK channels and consequently excite single VTA neurons (26). Interestingly, tipepidine, unlike methamphetamine and bupropion, failed to increase the level of dopamine in the NAc in rats with a mechanically lesioned VTA. We also preliminarily found that tipepidine, unlike methamphetamine, does not express behavioral sensitization in rodents when administered repeatedly (27).

The antidepressants developed thus far all inhibit monoamine transporters to increase the level of monoamines in the brain. In contrast to these drugs, tipepidine and cloperastine have a very weak action on the transporters and on various neurotransmitter receptors. Both drugs also have only a weak action on various channels other than GIRK channels, such as the TREK channels, which is considered to be a possible target for antidepressants. Apart from the above, Kobayashi et al. reported that many antidepressants developed thus far weakly inhibit GIRK-channel current when expressed in oocytes (28, 29) and suggested that the inhibitory action might be involved in the side effects of antidepressants (28, 29).

Taken together, these data suggest that tipepidine likely has a novel antidepressant-like action, showing an effect on depression that is resistant to treatment with preexisting antidepressants and showing a novel mechanism that at least partly involves inhibiting the GIRK channel in the brain.

4. Concluding remark

Prior to closing this review, I will mention another idea proposed by Luscher and Slesinger (3). They described in their excellent review that activation of GIRK channels causes analgesic action in rodents, introducing studies using GIRK channel-knockout mice. However, we found that tipepidine also has an analgesic action, although the drug does not activate GIRK channels. Antidepressants are also known to have analgesic action, probably by modulating monoamine levels in the brain. Thus, the action of tipepidine on pain is also likely to be due to increases in monoamine levels through the inhibition of GIRK channels in the brain.

As described in this review, however, antitussive drugs

Fig. 2. Schema showing excitation of monoamine neuron by GIRK-channel blockade with antitussives. Activation of GPCR (G protein-coupled receptor) with neurotransmitter causes activation of GIRK (G protein-coupled inwardly rectifying K⁺) channel to induce a resting or inhibited state of monoamine neuron. Blockade of GIRK channel by antitussive causes the activated state of monoamine neuron to increase the release of monoamine (neurotransmitter). To simplify, subunit constitution of G protein was not depicted in this schema.

such as tipepidine and cloperastine that have GIRK channel–inhibitory action ameliorate the symptoms of intractable brain diseases in various animal models. Although the mechanism remains to be studied, circumstantial evidence obtained from pharmacological and neurochemical studies suggests that the multiple and novel pharmacological actions of antitussives might be caused at least partly by inhibiting GIRK channels in the brain.

The findings obtained using GIRK channel–knockout mice (3) seem not to support our idea as described above. It is, however, important to note the basic findings on GIRK channels. GIRK channels are expressed as a homotetramer or heterotetramers of GIRK1 to GIRK4 subunits, are widely distributed, and are coupled to various GPCRs in the brain (3). Furthermore, the channels exist in different subcellular regions in neurons such as dendritic shafts and dendritic spine, in the postsynaptic density, and in the perisynaptic site (3). The affinity of GIRK channel for the $G\beta\gamma$ subunit of G protein seems to depend on the subunit components of GIRK channels (3). In addition, it is unknown as to whether a combination of GIRK-channel subunits is specific to each GPCR. The function of GIRK channels is also regulated by various factors (3). Whether GIRK channels in certain regions of the brain that are coupled to certain GPCRs are more susceptible to certain drugs is unknown. Thus, our working hypothesis regarding the mechanism of the pharmacological effect of antitussives is not discarded by the data from GIRK channel–knockout mice. Further studies are undoubtedly needed regarding the mechanisms of the multiple pharmacological actions of antitussives and on the physiology and pharmacology of GIRK channels. Nevertheless, it is a very intriguing idea that GIRK channels are a molecular target for developing new therapeutic drugs for intractable brain diseases and that the drugs possessing GIRK channel–blocking action, such as centrally acting antitussives, may be plausible candidates for such drugs.

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