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Effects of Ginseng Total Saponins and U-50,488H on Electrically Induced Twitch Responses of Mouse Vas Deferens

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Abstract□The effects of ginseng total saponins (GTS) on the action of U-50,488H, a κ-opioid receptor agonist, on the electrically induced twitch responses of mouse vas deferens were studied. U-50,488H (10^{-9}~10^{-5} M) inhibited the twitch contractions in a dose-dependent manner, which were caused by adenosine 5'-triphosphate (ATP) released from the stimulated sympathetic nerve, and this effect was antagonized by naloxone (10^{-6} M). GTS, which itself induced the inhibition of the twitch contractions, acted additively to U-50,488H, GTS and U-50,488H had no effect on the tension of the unstimulated organs. The contractions elicited by ATP were not affected by U-50,488H, but inhibited by GTS. These results suggest that U-50,488H suppressed the twitch contractions by the inhibition of neurotransmitter release from presynaptic nerve terminals via action on opioid receptor, but GTS, by inhibiting the action of the neurotransmitter on the smooth muscle.

Key words□Ginseng total saponins, U-50,488H, mouse vas deferens, opioid receptor, twitch contractions.

Introduction

It has been shown from *in vitro* studies that opioids exert a presynaptic inhibitory effect on excitatory neurotransmission in smooth muscle tissues such as the guinea pig ileum and the vas deferens of various species.[1] It was reported that mouse vas deferens interacted with ligands of δ-, κ- and μ-opioid receptor subtypes, although the δ-subtypes predominated in this tissue.[2] Thus, morphine inhibits the electrically induced contractions of mouse vas deferens via δ-subtypes.[2] A prototypic κ-agonist, ethylketocyclazocine, and endogenous κ-selective peptide, dynorphin, inhibit the electrically evoked contractions of this tissue, and it is reversed by naloxone, a nonselective opioid antagonist, suggesting that the effects of these compounds are mediated by the activation of κ-receptor subtype.[3,4]

Vas deferens of mammals have a marked arrangement of sympathetic innervation.[5] Electrical field stimulation of this sympathetic nerve induces the release of norepinephrine (NE) and ATP as cotransmitters, followed by contractions mediated by α_1-adrenergic and P_2-purinergic postsynaptic receptors, respectively.[6-10] Hughes *et al.*[11] demonstrated that morphine inhibited the electrically induced contractions of mouse vas deferens. They suggested that it was caused by the inhibition of NE release produced by sympathetic nerve stimulation. However, the possibility that ATP is involved in the contractions induced by electrical stimulation of brief low frequency has been suggested by Forsyth and Pollock.[12] In the preliminary report,[13] we elucidated that morphine inhibited the electrically induced contractions of mouse vas deferens by suppressing ATP release rather than NE in the experimental condition of brief low frequency stimulation (0.1 Hz, 1 ms duration).

Much attention has been paid to ginseng saponins because of their multiple pharmacological ac-

tions. Recently, Kim *et al.*[14-16] have demonstrated the effects of ginseng saponins on opioids-induced antinociception. Morphine- and U-50,488H-induced antinociception was prevented by pretreatment with GTS in mice. The development of tolerance to their antinociception was also inhibited by GTS. U-50,488 H displays antinociceptive action in a variety of assays using mice and rats, which is mediated by κ-opioid receptor subtype specifically.[17,18] By examining the effects of GTS on the regulation of neurotransmission mediated by specific opioid receptors *in vitro* experiments using peripheral tissues, we can elucidate not only the relationship between GTS and the specific opioid receptors but also the effects of GTS on the peripheral neurotransmission. We reported that morphine and GTS inhibited the electrically induced twitch responses of mouse vas deferens by distinct mechanisms.[13] In the present experiments, U-50,488H modulation of electrically induced contractions of mouse vas deferens was elucidated. And in order to characterize the interaction of GTS with κ-opioid receptor subtypes, the effects of GTS on the actions of U-50,488H on electrically induced contractions of mouse vas deferens was investigated.

Materials and Methods

The vas deferens isolated from mouse (ICR, 5~8 weeks suspended in a 5 m*l* organ bath containing Mg^{2+}-free[11] Krebs solution composed of (mM) NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $NaHCO_3$ 25 and glucose 11, and bubbled with 95% O_2-5% CO_2 at 37℃. The resting tension of the preparation was maintained at 200 mg.[19] After an equilibration period of 60 min, the electrical field stimulation of 1 ms duration was applied at 0.1 Hz with 80 V (Bioscience) through a pair of platinum electrodes. Contractions were recorded isotonically by a displacement transducer (Bioscience).

Cumulative concentration-response curves to GTS and U-50,488H were obtained following the stabilization of the contractile response to electrical field stimulation. The drugs were cumulatively added to the bath immediately after the response to each concentration of the drug was stabilized. Naloxone was applied 5 min before GTS and U-50,488H. To clarify th effect of GTS on the action of U-50,488 H, cumulative concentration-response curve to U-50,488H was obtained after stabilization of the responses to GTS in a concentration of 10 μg/m*l*. The effects of GTS and U-50,488H on the contractile responses to ATP or NE were observed by applying these drugs 5 min prior to ATP or NE. The results were expressed as percentages of control contractions without any drug treatment.

The sources of the drugs used were as follows: ATP, α,β-methylene ATP, NE bitartrate, U-50,488 H, tetrodotoxin, naloxone HCl and guanethidine sulfate were purchased from Sigma Chemical Co. (ST. Louis, MO) and prazosin HCl was obtained from Pfizer Co. and GTS was a gift from Korea Gineng & Tobacco Research Institute.

Results

Electrical field stimulation of isolated mouse vas deferens elicited individual phasic contractions of the rapid twitch type (Fig. 1). As shown in the preliminary report,[13] they were inhibited by tetrodotoxin, guanethidine and α,β-methylene-ATP, but were unaffected by prazosin, suggesting that they resulted from ATP released following postganglionic sympathetic nerve stimulation (data not shown).

1. Effects of GTS and U-50,488H on the twich contractions and antagonism by naloxone

GTS inhibited the twitch contractions of mouse

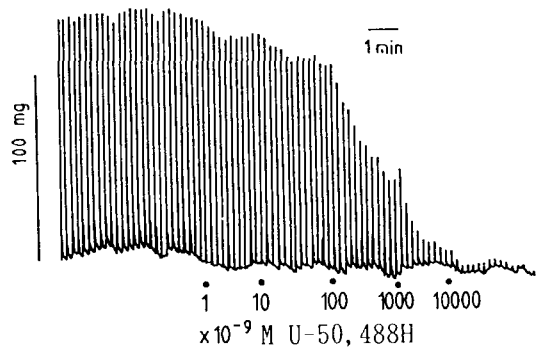

Fig. 1. Typical tracings of electrically elicited contractions of mouse vas deferens. The drug was added cumulatively at the time indicated by dots.

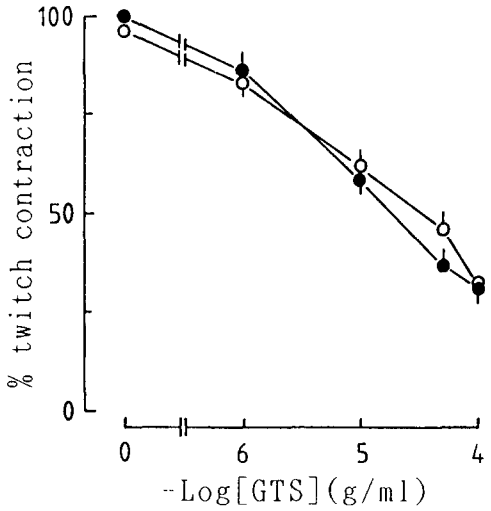

Fig. 2. Concentration-response curves of GTS on electrically induced twitch contractions of mouse vas deferens in the presence (○–○) o absence (●–●) of naloxone. Naloxone (10^{-6} M) was added 5 min before GTS. Amplitude of the contraction just before addition of GTS was taken as 100%. The values are means± S.E. of 7 to 8 preparations.

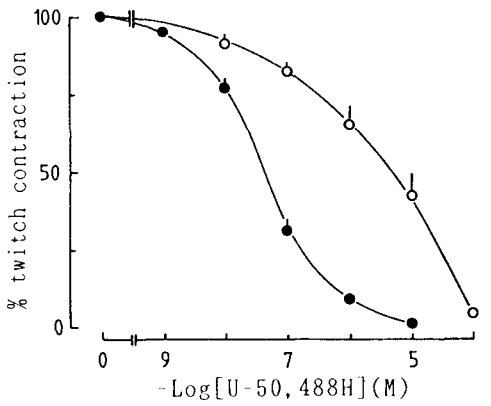

Fig. 3. Concentration-response curves of U-50,488H on electrically induced twitch contractions of mouse vas deferens in the presence (○–○) or absence (●–●) of naloxone. Naloxone (10^{-6} M) was added 5 min before U-50,488H. Amplitude of the contractions just before addition of U-50,488H was taken as 100%. The values are means± S.E. of 7 to 8 preparations.

vas deferens in a concentration-dependent manner and the contractions were reduced by 70% by 100 μg/ml of GTS (Fig. 2). The contractions were not

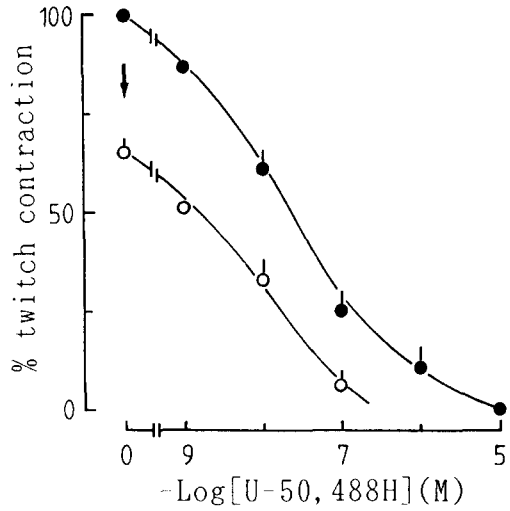

Fig. 4. Effect of GTS on the concentration-response of U-50,488H on electrically induced twitch contractions. Concentration-response curves of U-50,488H were obtained either after the treatment (○–○) or untreatment (●–●) of 10 μg/ml GTS. The symbol of arrow represents the reduction of the control contractions by 10 μg/ml GTS. The values are means± S.E. of 8 preparations.

restored to the control level even after intensive washings after applying a concentration of GTS greater than 100 μg/ml. U-50,488H (10^{-9}~10^{-5} M) caused dose-dependent inhibition of the twitch response (Fig. 3). The value of IC_{50} for U-50,488H was determind to be 40×10^{-9} M. The inhibition by U-50,488H was readily reversible on washings. Naloxone, a nonselective opioid receptor antagonist, at 10^{-6} M did not significantly affect the control contractions (96± 4%). The pretreatment with 10^{-6} M naloxone caused the concentration-response curve of U-50,488H to shift to the right (Fig. 3).

And naloxone at the same concentration completely reversed 10^{-6} M U-50,488H-induced inhibition of the twitch response when applied after U-50,488 H treatment (data not shown). However, the inhibition of the twitch contrctions by GTS was not affected either by the pretreatment with naloxone or by naloxone applied after GTS treatment (Fig. 2).

2. Effect of GTS on the U-50,488H-induced modulation of the twitch contractions

As shown in Fig. 4, GTS (10 μg/ml) reduced the

Table 1. Effects of GTS and U-50,488H on contractions elicited by ATP or NE in unstimulated mouse vas deferens

Additions	Contractions (%)	
	ATP	NE
None	100	100
GTS (10^{-5} g/ml)	81± 9	74± 6
GTS (10^{-4} g/ml)	29± 7	79± 10
U-50,488H (10^{-6} M)	97± 9	89± 10

Control value (100%) of ATP (10^{-6} M)- or NE (10^{-6} M)-induced contractions was the mean of three trials which were done every 10 min. The values are means± S.E. of 4 to 5 preparations.

control contractions by 34± 4%, but GTS did not affect the U-50,488H-inducd ingibition of the twitch contractions. This fact suggests that GTS acts additively to the effect of U-50,488H and inhibits the twitch contractions by a mechanism different from that of U-50,488H.

3. Effects of GTS and U-50,488H on NE- or ATP-induced contractions

GTS and U-50,488H did not cause any distinct change in basal tension of the unstimulated preparations over the concentration ranges used in the present study. U-50,488H at the concentration of 10^{-6} M did not affect the contractions elicited by exogeneously applied NE (10^{-6} M) or ATP (10^{-6} M) (Table 1). GTS at 100 μg/ml slightly inhibited the contractions induced by NE, but greatly inhibited the ATP-induced contraction (Table 1).

Discussion

Mouse vas deferens which interacts with the ligands of δ-, κ-, and μ-opioid receptors[2] is a suitable model for the prediction of the agonist and antagonist properties of narcotic analgesics. U-50,488H is a selective compound at κ-receptor in *in vitro* and *in vivo* studies.[17,18,20-22]. In the present experiments, U-50,488H inhibited the electrically induced contractions of mouse vas deferens in a dose-dependent manner and this inhibitory dose-response curve was shifted to the right by naloxone pretreatment. Although a specific κ-receptor antagonist is not available, regarding the facts that U-50,488H is a selective κ-ligand and naloxone at higher concentrations also antagonizes the action of compounds having κ-affinity, we can conclud that U-50,488 H produced the inhibitory effect via the action on *k*-receptor.

U-50,488H did not modify the basal tension and ATP- as well as NE-elicited contractions. As described in results, ATP seems to be responsibl for the electrically induced twitch contrctions under the present experimental conditions. So it is most likely that U-50,488H attenuates the twitch contractions by suppressing ATP release presynaptically.

GTS significantly inhibited the twitch contractions in a dose-dependent manner. The inhibitory effect of GTS on the twitch contractions was not antagonized by naloxone. We reported the same result in the preliminary experiment.[13] And the interaction between GTS and U-50,488H on the twitch contractions was also examined in this study. GTS did not affect the inhibitory action of U-50,488H on the twitch contractions but acted additively to the effect of U-50,488H because of the inhibition by itself of the twitch contractions. These results indicate that GTS inhibits the twitch contractions by a mechanism different from that of U-50,488H without being mediated by opioid receptors. This is an additional finding that GTS inhibits the twitch contractions of mouse vas deferens by a nonopioid mechanism. In the preliminary report,[13] we also elucidated that GTS did not affect the inhibitory action of morphine on the twitch contractions.

GTS inhibited the exogeneous ATP-elicited contractions and the rate was closely correlated to the inhibitory rate on the electrically induced twitch contractions. Therefore, it is suggested that GTS inhibited the twitch contractions by inhibiting the action of ATP, which was released from the sympathetic postganglionic nerve stimulation, on the smooth muscle via P_2-purinoceptor.

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