Decreasing Effects by Glycyrrhizin and Paeoniflorin on Intracellular Ca\(^{2+}\)-Aequorin Luminescence Transients with or without Caffeine in Directly Stimulated-Diaphragm Muscle of Mouse

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Abstract—Effects of glycyrrhizin (GLR) and paeoniflorin (PF), depolarizing neuromuscular blockers, on intracellular Ca\(^{2+}\)-aequorin luminescence in the presence of or the absence of caffeine were investigated, using directly stimulated diaphragm muscle of mouse. Both GLR and PF decreased Ca\(^{2+}\)-aequorin luminescence. The caffeine-induced increase in Ca\(^{2+}\)-aequorin luminescence was inhibited by GLR, but not by PF. These results demonstrated that GLR and PF decreased intracellular Ca\(^{2+}\); GLR suppressed Ca\(^{2+}\)-releasing from the site of action of caffeine, but PF did not.

Glycyrrhizin (GLR) and paeoniflorin (PF) are principle components of licorice roots and paeony roots, respectively. In the previous paper, we found that PF and GLR caused an inhibitory combined effect on twitch tensions of nerve-stimulated skeletal muscle (1), depolarized resting membrane potentials and depressed acetylcholine potentials (2).

Recently, the measurement of aequorin-Ca\(^{2+}\) transients has been reported in several tissues (3–5). We investigated fundamental properties of Ca\(^{2+}\) transients in directly stimulated diaphragm muscles of mouse (6), and we found that 1) procaine suppressed both Ca\(^{2+}\) transients themselves and caffeine-induced increase of Ca\(^{2+}\) transients in directly stimulated diaphragm muscle of mouse, 2) dantrolene suppressed Ca\(^{2+}\) transients, but not the caffeine-induced one, and 3) succinylcholine (SuCh), a depolarizing blocker, also suppressed Ca\(^{2+}\) transients, but d-tubocurarine did not (6). In the present study, we investigated whether GLR or PF, also depolarizing blockers like SuCh, affect intracellular Ca\(^{2+}\) transients in mouse diaphragm muscle.

Male ddY mice weighing 30–36 g (7–8 weeks) were decapitated and bled. Diaphragm muscles with tendon were removed and cut into strips 5 mm wide. One end of the muscle was connected to an isometric transducer for mechanical recording. The other end was pinned to a rubber plate. The resting tension was adjusted to 100 mg. A modified Krebs' solution of the following composition (mM) was used: NaCl, 122; KCl, 5.9; CaCl\(_2\), 2.5; MgCl\(_2\), 1.2; NaHCO\(_3\), 15.5; and glucose, 11.5. The bath solution was maintained at 37±1°C and equilibrated with 95% O\(_2\) and 5% CO\(_2\). The solution of the following drugs: caffeine (Nakarai), glycyrrhizin monoammonium salt (Mino-phagen Co.) and paeoniflorin were perfused over the bath. The techniques to measure aequorin-Ca\(^{2+}\) transients described by Blinks, Rudel and Taylor (2) were slightly modified. The aequorin (Mayo Clinic) solution (1 mg/ml) containing 150 mM KCl, 5 mM HEPES (pH 7.45) and 10 \(\mu\)M EGTA was filtered through a milipore filter (0.05 \(\mu\)m). The aequorin solution was taken into the glass capillary micropipette (10–30 \(\mu\)l). While monitoring membrane potential, the aequorin solution was injected 1–2 times per each fiber into the cytosols of 40–50 fibers through the micropipette under the condition of 3–8
kg/cm², 1–2 sec. The aequorin luminescence in the injected area was measured using a photon counter (slightly modified C767, Hamamatsu Photonics) and a photomultiplier tube (R464, Hamamatsu Photonics) attached to an acrylic optical fiber. The open
gate time of the photon counter was set at 10 msec, and the closing gate time at 20 μsec. For the wave form analysis, six aequorin luminescences were averaged using a signal processor (7T07A, San-ei). The partition stimulating method described by Abe and Tomita (7) was used for applying the supramaximal stimulation (duration 50 msec, 0.1 Hz). The stimulation currents were applied to a 1 mm wide area in the muscle segment by Ag-AgCl plates and was monitored by another pair of Ag-AgCl electrodes 1 mm

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**Fig. 2. Effects of 2.1 mM peoniflorin (PF) with or without 5 mM caffeine on aequorin luminescence and twitch tension in mouse diaphragm muscle stimulated directly.**

A: Typical recordings of aequorin luminescence (upper) and twitch tensions (lower) with 2.1 mM PF alone or in the presence of 5 mM caffeine. The solution of each drug was perfused as in Fig. 1A.

B: Typical fast traces of averaged records of six aequorin luminescences (upper) and monitored stimulation (lower) during 1 min. a: Control before the application of PF, b: 7-8 min after the application of PF, c: 2.5-3.5 min after the addition of caffeine in the presence of PF, d: control before the application of caffeine, and e: 3-4 min after the addition of caffeine alone.

C: The logarithmic % luminescence peak amplitude and % twitch tension measured every 10 sec and replotted from Fig. 2A. The mean values (●) were plotted as in Fig. 1C. Open circles represent the mean values at a single time point in different experiments (n=3), and the vertical lines show S.E.M.
apart as stimulation voltage (V/mm). The details of this method were described in the previous paper (6). Logarithmic change of the peak luminescence of aequorin-Ca\(^{2+}\) transients corresponding to those of evoked twitch tension was plotted. The concentration used of caffeine, GLR and PF had no effects on the light luminescence of aequorin.

GLR (1.2 mM) decreased Ca\(^{2+}\)-aequorin luminescence amplitudes after an initial increase, tended to shorten the duration, and reduced the caffeine-induced increase in the luminescence amplitude, being accompanied concurrently by the decrease in directly stimulated twitch tensions (Fig. 1A, B and C). The gradual attenuation of control signals and caffeine-induced increase of the luminescence and twitch tension have been previously reported (6). PF (2.1 mM) also decreased Ca\(^{2+}\)-aequorin luminescence amplitudes and broadened the luminescence, although this decreasing effect of PF is weaker than that of GLR (Fig. 2A, B and C). However, even in the higher concentration (12.5 mM) of PF, it did not affect the caffeine-induced increase in the luminescence amplitude.

The above results demonstrated that either GLR or PF decrease the electrically stimulated increase in intracellular free Ca\(^{2+}\) concentrations. The decreasing amounts in the response by GLR or PF became much larger than the deterioration amounts without drugs. The decreasing effect of GLR or PF alone on the luminescence and twitch tension became constant at 10 min after the application. The luminescence signal became smaller and broader by PF. PF or GLR depolarized the resting membrane potential to only a few mV. Decrease in Ca\(^{2+}\) transients are not attributed to these small amounts of depolarization.

The effects of both drugs on caffeine-induced increase in Ca\(^{2+}\) transients were quite different. GLR seems to depress a Ca\(^{2+}\) releasing process related to the site of action of caffeine. In this respect, the effect of GLR seems to be similar to that of procaine. In another previous paper, we reported that GLR elongated the duration of muscle action potential (8). In skeletal muscle myotubes, the presence of Ca\(^{2+}\)-activated K\(^+\) channels were recently reported by Barrett et al. (9). As one of the reaction mechanisms by GLR, the present results, therefore, suggest the predictive link to Ca\(^{2+}\)-activated K\(^+\) channels.

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References
1. Kimura, M., Kimura, I., Takahashi, K., Muroi, M., Yoshizaki, M., Kanaoka, M., and Kitagawa, I.: Blocking effects of blended paeoniflorin or its related compounds with glycyrrhizin on neuromuscular junctions in frog and mouse. Japan. J. Pharmacol. 36, 275-282 (1984)
2. Kimura, M., Kimura, I., and Nojima, H.: Depolarizing neuromuscular blocking action induced by electropharmacological coupling in the combined effect of paeoniflorin and glycyrrhizin. Japan. J. Pharmacol. 37, 389-399 (1985)
3. Blinks, J.R., Rudel, R. and Taylor, S.R.: Calcium transients in isolated amphibian skeletal muscle fibers. Detection with aequorin. J. Physiol. (Lond.) 277, 291-323 (1978)
4. Eusebi, F., Miledi, R. and Takahashi, T.: Calcium transient in mammalian muscles. Nature 284, 560-561 (1980)
5. Eusebi, F., Miledi, R. and Takahashi, T.: Aequorin-calcium transients in frog twitch muscle fibers. J. Physiol. (Lond.) 340, 91-106 (1983)
6. Kimura, I., Kimura, M. and Kimura, M.: Modification by dantrolene, procaine and suxamethonium of caffeine-induced changes in aequorin luminescence transients and twitch tensions of directly-stimulated diaphragm muscle of mouse. Br. J. Pharmacol. (1986) (in press)
7. Abe, Y. and Tomita, T.: Cable properties of smooth muscle. J. Physiol. (Lond.) 196, 87-100 (1968)
8. Kimura, M., Nojima, H. and Kimura, I.: Effect of glycyrrhizin on electrophysiological K\(^+\) in mouse diaphragm muscles. Folia Pharmacol. Japon. 82, 12P (1983) (in Japanese)
9. Barrett, J.N., Barrett, E.F. and Dribin, L.B.: Calcium-dependent slow potassium conductance in rat skeletal myotubes. Dev. Biol. 82, 258-266 (1981)