Depolarizing Effects of Glycyrrhizin-Derivatives Relating to the Blend Effects with Paeoniflorin in Mouse Diaphragm Muscle

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Abstract—Glycyrrhizin (GLR) and its newly synthesized derivatives, deoxoglycyrrhetol dihemisuccinate (I), deoxoglycyrrhetol dihemiphthalate (II), and the related compounds, carbenoxolone and glycyrrhetinic acid hemiphthalate (III), were assayed with or without paeoniflorin (PF) in mice diaphragm muscles. GLR-derivatives per se blocked the nerve-stimulated twitch tensions with the following order of potencies: carbenoxolone=III>II=I>GLR. The potencies paralleled the extent of muscle depolarization except in the case of I. GLR and I only increased muscle conductance. The blocking effects by GLR-derivatives per se on twitch responses are, therefore, can be explained by depolarization of muscle membranes and not by membrane conductance change. When carbenoxolone, II or GLR were blended with PF, the potentiation of the blocking effects observed may be related to the muscle depolarization rather than by the increase in membrane conductance.
standard glass microelectrodes filled with 3 M KCl (tip resistance of 5–15 MΩ). R.m.p. was measured using a microelectrode amplifier (Diamechical; DPZ-30, DPS-180, and DPS-165B) with a single micro-electrode (6, 7). Membrane potentials were held at −90 mV and then were voltage-clamped from −90 mV to −120 mV (duration 300 msec, sampling rate 5 KHz) through the same electrode. The inward currents were recorded on Visilight (San-ei, 5M21).

GLR, I, II and carbenoxolone showed the concentration-dependent inhibition of indirectly stimulated-twitch response (Fig. 1). Compound III in a concentration more than 0.1 mM increased the resting tension, and so % inhibition was not determined.

PF did not show this effect in concentrations up to 19 mM (data not shown). Blocking effects of GLR, II and carbenoxolone were potentiated by the combination with the same molar ratio of PF, but those of I and III were not (Fig. 1). GLR decreased r.m.p. as shown previously (5), and it increased membrane conductance in the concentration range of 0.12–1.2 mM, whereas GLR-derivatives except GLR had only an alternative effect. Carbenoxolone (0.15–0.2 mM), compound II (0.1–0.5 mM) and III (0.1–0.2 mM) decreased r.m.p. (Fig. 2), but none of them significantly increased membrane conductance (data not shown). Compound I (0.5–1 mM) increased membrane conductance (Fig. 2), but it did not significantly decrease r.m.p. (data not shown).

The twitch inhibition by GLR-derivatives in Fig. 1 paralleled the extent of membrane depolarization in Fig. 2 except in the case of compound I. The above results indicate that the blocking effects of GLR-derivatives per se on twitch responses are closely explained by depolarization of muscle membranes, but not by membrane conductance change.

The potencies of the twitch inhibition and the muscle depolarization are possibly attributed to the presence of the carboxylic acid in ring E and the carbonyl group in ring C of GLR. Shibata (2) reported that the presence of a hydroxymethyl group in the oleanane-type triterpenoids was a significant factor for their pharmacological activities. In the blocking activities on twitch responses, however, carbenoxolone and III which have the carboxylic acid structure in ring E were more potent than I and II having the hydroxymethyl structure.

The effects of GLR blended with PF were accompanied with muscle depolarization (5). The inhibitory effects of II and carbenoxolone were also potentiated by the combination of PF. These compounds depolarized the muscle membrane, but did not increase the membrane conductance.
conductance. Hence, the blend effects of GLR-derivatives as well as GLR on nerve-stimulated twitch response may be explained by the membrane depolarization rather than by the increase in membrane conductance.

Fig. 2. Effects of GLR-derivatives on resting membrane potential (r.m.p.) and resting membrane conductance of mouse diaphragm muscles. R.m.p. changes (Δ r.m.p.) by GLR, compound II, III and carbexolone (A) and resting membrane conductance changes (Δ conductance) by GLR and compound I (B) of diaphragm muscles at 30–90 min after the addition of GLR-derivatives were plotted against the log concentration. R.m.p. changes by compound I, and conductance changes except by GLR and compound I were not observed. The values are means±S.E.M. (n=3–18). *(P<0.05) and **(P<0.01) indicate significant differences from Δ values without GLR-derivatives in the paired t-test.

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