DDB suppresses the rate of release of creatine kinase from muscle accelerated by dimethylformamide *in vitro*

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ABSTRACT

The rate of release of creatine kinase (CK) from the extensor digitorum longus (EDL) muscle of hamsters was estimated *in vitro*. In oxygenated Krebs-Ringer solution, the EDL muscle was electrically stimulated to produce continuous twitch response. The rate of CK release gradually increased to the level 6 times higher than the initial value after 3 h of incubation. When dimethylformamide was added to the medium, the rate of CK release was further accelerated. Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2,2'-dicarboxylate (DDB), an intermediate product in the process of synthesizing Schizandrin C, suppressed the rate of CK release accelerated by dimethylformamide. The mechanism and significance of the present observation were discussed.

The skeletal muscle suspended in physiological saline releases some of the sarcoplasmic enzymes into the medium (3). The release can be increased when the permeability of sarcolemma is altered ('leaky' membrane theory) (11) or sarcolemma is physically disrupted ('holey' membrane theory) (1, 6), as postulated for explaining the genesis of Duchenne muscular dystrophy. However, there are few compounds capable of inhibiting the release of sarcoplasmic enzymes (2, 7, 8). In the present experiment, we have studied the release of creatine kinase (CK) from the extensor digitorum longus (EDL) muscle of the hamster *in vitro*. Dimethylformamide, an organic solvent, has a property to accelerate the rate of CK release. Seven compounds isolated from *Fructus Schizandrae chinensis*, a traditional Chinese medicine, are named Schizandrin (Sin) A, B and C, Schizandrol (Sol) A and B,

and Schizandrone (Ser) A and B. Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2,2'-dicarboxylate (DDB) is an intermediate product in the process of synthesizing Sin C (5). DDB was found to suppress the rate of CK release accelerated by dimethylformamide.

The experiments were performed with EDL muscles isolated from 9 male golden hamsters aged 14-16 weeks, and weighed about 100 g. The method used in this study was almost the same as in our previous study with the dystrophic hamster (15). The EDL muscle was preincubated for 1 h in a medium bubbled with 95% O₂-5% CO₂ to attenuate initial CK release and to facilitate free access of dimethylformamide or DDB. The EDL muscle was electrically stimulated to produce continuous twitch response (square wave pulse; 1 Hz, 30 V, and 10 msec). The muscle was kept apart from the electrodes to avoid injury during contraction. The rate of CK release was expressed as CK activity divided by the muscle wet weight per 30 min (U/30 min/g).

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Fig. 1 Rate of release of creatine kinase (CK) from the extensor digitorum longus muscle of hamsters in the oxygenated Krebs-Ringer solution (KRS; open circles, $n = 4$), Krebs-Ringer solution containing dimethylformamide (DMF; closed circles, $n = 5$), and Krebs-Ringer solution containing DDB dissolved in dimethylformamide (DMF plus DDB; triangles, $n = 5$). Mean values and SD are plotted. The values were larger in DMF than in KRS; the difference was significant at $P < 0.005$ (1.5 h), at $P < 0.005$ (2 h), at $P < 0.025$ (2.5 h), and at $P < 0.005$ (3 h). Addition of DDB in DMF significantly decreased the rate of CK release compared to that in DMF alone: $P < 0.005$ (1.5 h), $P < 0.01$ (2 h), $P < 0.005$ (2.5 h), and $P < 0.005$ (3 h).

DDB was dissolved in 1% dimethylformamide in Krebs-Ringer solution to 0.0025% (w/v). Krebs-Ringer solution, Krebs-Ringer solution containing dimethylformamide, Krebs-Ringer solution containing DDB dissolved in dimethylformamide will be referred to as KRS, DMF, and DMF plus DDB, respectively. We have excluded the possibility that dimethylformamide or DDB exerts a direct effect on the activity of CK during storage for 3 h at 4°C prior to analysis. It is also unlikely that DDB directly reacts with dimethylformamide, because the incubation of 0.0025% DDB in Krebs-Ringer solution containing 1% dimethylformamide for 1 h at 37°C did not show any evidence for interaction of two compounds when examined by thin-layer chromatography (data not shown). Student's *t* test was used for statistical analysis of the results.

Fig. 1 shows the rate of CK release from the EDL muscle *in vitro*. In KRS, the rate of CK

release from the muscle was unchanged for 1.5 h, but gradually increased thereafter to reach the level 6 times as high as the initial value after 3 h. In DMF, the initial value of CK release was 1.0 ± 0.7 which was already 2.5 times higher than the value in KRS. This may be attributable to an increase in leakage of CK due to dimethylformamide during preincubation. The rate of CK release abruptly increased after 1 h and reached the level 18 times as high as the initial value after 3 h. There is a significant difference between KRS and DMF groups after 1.5 h ($P < 0.005$), indicating that dimethylformamide may have damaged the sarcolemma and accelerated the release of CK from the sarcoplasm. Addition of DDB in DMF (DMF plus DDB) markedly suppressed the rate of CK release accelerated by dimethylformamide to the level in KRS. There is a significant difference between DMF and DMF plus DDB groups after 1.5 h ($P < 0.005$), indicating that DDB antagonizes the effect of dimethylformamide with respect to the CK release from the sarcoplasm.

The isolated, intact muscle incubated in a variety of physiological solutions steadily releases sarcoplasmic enzymes (3, 4, 9, 14, 17, 18, 20). The mechanism underlying the enzyme release from intact muscle is not clear, but both exocytosis and passive diffusion have been proposed (11, 13, 14). The rate of leakage of enzymes from muscle may indicate the property of membrane or other structures that normally prevent or minimize the leakage (3). The rate of release of sarcoplasmic enzymes is modified under various conditions, including hypoxia (4), strong muscle contraction (4), excess in potassium ions (4), chronic denervation (4, 9), increased calcium concentration (10, 12), metabolic inhibitors (iodoacetate, 2,4-dinitrophenol and cyanide) (10, 19, 20), membrane depolarization (19), repetitive stimulation (19), mechanical injury (14), freezing and thawing (3), agents which affect phospholipids (phospholipases A and C) (3, 16), and detergents (sodium dodecylsulfate and Brig 58) (3, 14).

Skeletal muscle of dystrophic mice, hamsters and chickens may release more sarcoplasmic enzymes into the circulatory blood than control animals (4, 15, 21). Concerning the mechanism of the extraordinary high CK activity in the serum of patients with Duchenne muscular dystrophy, two main explanations have been given. First, the enzyme protein can be released through the

disrupted sarcolemma of necrotic fibers ('holey' membrane theory) (1, 6). Second, the sarcolemma may be functionally abnormal and the sarcoplasmic enzymes leak out through the membrane ('leaky' membrane theory) (11). These two explanations are not mutually exclusive. Physical disruption combined with altered permeability of the sarcolemma may explain the excessive release of sarcoplasmic enzyme proteins (11).

Although prednisolone, diethylstilbestrol and penicillamine are known to decrease the release of sarcoplasmic enzymes from the isolated intact muscles (2, 7, 8), another new drugs with a similar property have been searched. DDB is an intermediate product in the process of synthesizing Sin C which is one of seven compounds isolated from *Fructus Schizandrae chinensis*. *Fructus Schizandrae chinensis* is reported to be effective in the treatment of viral and chemically (carbon tetrachloride and thioacetamide) induced hepatitis, especially in lowering the elevated serum GOT and GPT levels. DDB exerts the same effect as *Fructus Schizandrae chinensis* in chemically induced hepatitis (5). In the present experiment, DDB was dissolved in dimethylformamide, because of its insolubility in various physiological solutions. Dimethylformamide, an organic solvent, may dissolve the sarcolemmal lipids and damage the sarcolemmal structure. We consider that antagonistic action of DDB against dimethylformamide may be associated with a direct protection of the sarcolemma from disruption by dimethylformamide, but further study is obviously needed to define the site of action of dimethylformamide and DDB in the muscle cell.

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