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A G-protein of sarcoplasmic reticulum of skeletal muscle is activated by caffeine or inositol trisphosphate

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Here we show the activation of G-protein by inositol trisphosphate (IP_3) or caffeine in sarcoplasmic reticulum (SR) of skeletal muscle and the consequent existence of a common mechanism of Ca^{2+} release from SR induced by caffeine and by IP_3. (i) Indomethacin inhibits Ca^{2+} release induced by IP_3 or caffeine. (ii) PGE_1 does not induce Ca^{2+} release itself, but does stimulate Ca^{2+} release induced by IP_3, or caffeine, from SR. (iii) Forskolin stimulates both types of Ca^{2+} release. The inhibitory effect of indomethacin on both forms of Ca^{2+} release, and the stimulatory effect of PGE_1 and forskolin on either Ca^{2+} release suggest that there exists a common mechanism between IP_3- and caffeine-induced Ca^{2+} release. (iv) Caffeine or IP_3 activates G-protein via inhibition of a GTPase activity. (v) Indomethacin itself inactivates this G-protein by stimulation of a GTPase activity and reverses the activation of G-protein induced by IP_3 or caffeine. (vi) PGE_1 competes with the inhibitory effect of indomethacin on GTPase activity and PGE_1 itself activates G-protein through inhibition of GTPase activity. From these results, it could be suggested that caffeine or IP_3 induces Ca^{2+} release from the SR via activation of G-protein, which affects the Ca^{2+} channel and cAMP which seems to affect G-protein via A-kinase.

G-protein; Ca^{2+} release; Caffeine; Inositol phosphate; (Sarcoplasmic reticulum, Skeletal muscle)

1. INTRODUCTION

It is well known that guanine nucleotide-binding proteins (G-proteins) serve as membrane-bound transducers of chemically and physically coded information [1]. Some recent reports lend more credence to the exciting possibility that G-proteins may exert direct control over the function of ion channels [2-6].

Recently, Scherer et al. [7] reported that G-protein exists in the SR of skeletal muscle. This SR shows the important physiological functions of Ca^{2+} release and uptake. We were therefore interested in clarifying the role of G-protein in this Ca^{2+} release and designed the present experiments accordingly.

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2. MATERIALS AND METHODS

All experiments were carried out at room temperature (22-24°C). Ca^{2+} uptake by and Ca^{2+} release from isolated SR fractions were continuously monitored by measurement of the difference absorption of murexide between 542 and 507 nm with a dual-wave spectrophotometer (Shimadzu UV 3000) at 15°C.

Ca^{2+} release: SR fractions were isolated from frog (*Rana*) leg skeletal muscles and purified by differential and density gradient centrifugation [8]. SR fractions were resuspended in 0.3 M sucrose, 10 mM Tric-HCl buffer (pH 7.5). Ca^{2+} uptake and Ca^{2+} release were continuously monitored by measurement of the difference absorption of murexide between 542 and 507 nm with a dual-wave spectrophotometer (Shimadzu UV 3000) at 15°C. The reaction medium was composed of 0.1 M KCl, 20 mM Tris-maleic acid (pH 6.8) buffer, and 1 mM ATP in 3 ml. 0.4 mg protein/ml SR fraction and 0.25 mM murexide were also added. The reaction was started by the addition of 30 μl of 30 mM $CaCl_2$. Subsequently, 3 mg powdered caffeine was added. When SR was added to the reaction medium, the resting free calcium level was 15 ± 0.5 μM ($n = 6$).

GTPase activities: the reaction medium comprised 0.15 M KCl, and 50 mM Tris-HCl buffer (pH 7.5) in 1 ml at 37°C. Since Kent et al. reported that 100 μM GTP induced the receptor form to become of low affinity [9], suggesting that G-protein

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adopts an active form which binds GTP exchanged from GDP according to the mechanism of receptor–G-protein interaction [10], we used high concentrations of GTP (250 μM) in order to observe a true GTP-hydrolysis step and also to avoid agonist stimulation of exchange steps between GDP and GTP. Reaction time was 10 min. In control experiments, linearity of GTPase activities was observed within 10 min. Specific activities of GTPase in controls were 120 ± 40 pmol/mg protein per min ($n = 6$). Liberated phosphate from GTP hydrolysis was measured according to Martin and Doty [11]. Non-specific nucleotide triphosphatase was inhibited by the addition of 50 mM adenosine 5′-(β,γ-imido)triphosphate (App(NH)p).

3. RESULTS AND DISCUSSION

Since we observed that indomethacin induced skeletal muscle fatigue (unpublished), we studied the effect of indomethacin on an excitation-contraction (EC) coupling system, since the mechanism of fatigue at the level of single skeletal muscle fibers seems to be related to the EC coupling system [12]. We then studied the effect of indomethacin on Ca^{2+} release from the SR.

It is known that Ca^{2+} release from SR is induced by IP_3, or caffeine. Hence, we studied the effect of indomethacin on Ca^{2+} release induced by IP_3 or caffeine from isolated SR. Fig.1 shows the Ca^{2+} release from SR. IP_3 and/or caffeine induced Ca^{2+} release, whereas indomethacin (10 μM) caused complete inhibition of both forms of Ca^{2+} release; PGE_1 (20 ng/ml) gave rise to a high degree of stimulation of these forms of release, but did not induce such release when present alone. This result, however, suggested the possibility that calcium contamination of the agents (especially IP_3) induced the Ca^{2+} release. We thus prepared samples of the above agents which contained EGTA at 2-fold greater concentration than that of the agent, and determined whether any differences occurred between the effect of the agents in the presence and absence of EGTA. We observed no difference in this case.

Since PGE_1 is known to increase the concentration of cyclic AMP (cAMP) via the activation of the PGE_1 receptor [13] and because Ong and Steiner [14] reported that the cAMP system exists predominantly in the SR and sarcolemma of skeletal muscle, we studied the effect of forskolin, which stimulates the activity of adenylate cyclase. As clearly shown in fig.1, forskolin greatly stimulated both forms of Ca^{2+} release.

The effect of cAMP on Ca^{2+} release from SR has

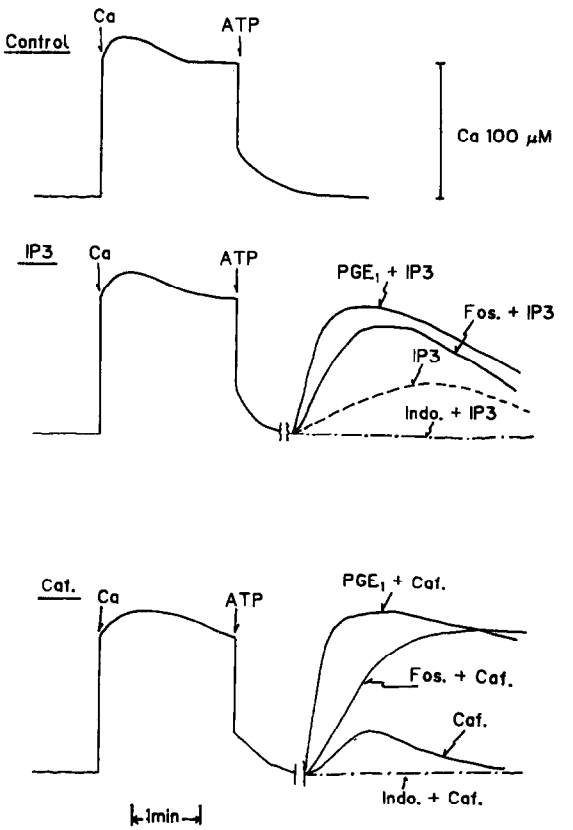

Fig.1. PGE_1 stimulation and indomethacin inhibition of Ca^{2+} release by IP_3 and/or caffeine from isolated SR fractions. IP_3 (2.5 μM) and caffeine (5 mM) induced Ca^{2+} release and the addition of PGE_1 (20 ng/ml) before IP_3 and caffeine promoted these forms of release. Forskolin (4 μM) greatly stimulated Ca^{2+} release induced by IP_3 and/or caffeine. Addition of indomethacin (10 μM) before IP_3 and caffeine completely inhibited both forms of Ca^{2+} release. Similar results were obtained using three different SR preparations. Ca, 100 μM $CaCl_2$; ATP, 0.5 mM; IP_3, inositol trisphosphate, 2.5 μM; Fos., forskolin, 4 μM; Caf., caffeine, 5 mM; Indo., indomethacin, 10 μM.

been previously examined by Meissner [15]. According to his results, the effect of cAMP on Ca^{2+} release was lower than that of AMP-PCP. It seems that cAMP acts from the inside of SR.

From our results on the PGE_1 requirement for both forms of Ca^{2+} release (IP_3- and caffeine-induced) and the stimulation by forskolin of both, it can be concluded that there exists a common mechanism between IP_3- and caffeine-induced Ca^{2+} release.

We next addressed the question as to indomethacin inhibits both forms of Ca^{2+} release (one

being induced by IP_3, the other by caffeine). In other words, why does PGE_1 stimulate both forms of Ca^{2+} release? Pike and Lefkowitz [13] reported that PGE_1 and β-adrenergic receptors share a common pool of guanine nucleotide regulatory protein (G-protein [1]). Based on their proposals and according to the suggestion of Tolman and Partridge [16], who reported that indomethacin showed an antagonistic effect to PGE_1 on gerbil colon contraction induced by PGE_1 and suggested the direct interaction between indomethacin and PGE_1 at common sites, indomethacin appears to act on this G-protein to inhibit Ca^{2+} release induced by IP_3, or caffeine, and contrastingly, PGE_1 also seems to act on this G-protein to stimulate Ca^{2+} release induced by IP_3, or caffeine. In fact, at less than 25 μM, indomethacin stimulated the GTPase activity of SR membrane, suggesting that indomethacin stimulation caused transformation of G-protein from an active into an inactive form (fig.2). In contrast, caffeine or IP_3 inhibited this GTPase activity, suggesting that caffeine or IP_3 maintained the G-protein in an active form (figs 3,4).

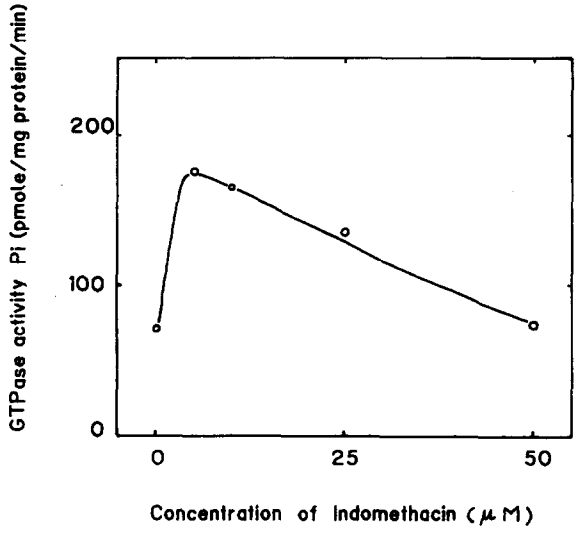

Fig.2. Effects of indomethacin on GTPase activity of SR membrane. SR fractions were the same as that of fig.1. Reaction medium comprised 0.15 M KCl, 50 mM Tris-HCl buffer (pH 7.5) in 1 ml. GTP concentration was 250 μM and the reaction temperature, 37°C. Reaction time was 10 min. Liberated phosphate from GTP was measured according to Martin and Doty [11]. Nonspecific nucleotide triphosphatase was inhibited by addition of 50 mM adenosine 5'-(β,γ-imido)triphosphate (App(NH)p). In the control experiment, liberated phosphate was not detected from IP_3 under these conditions.

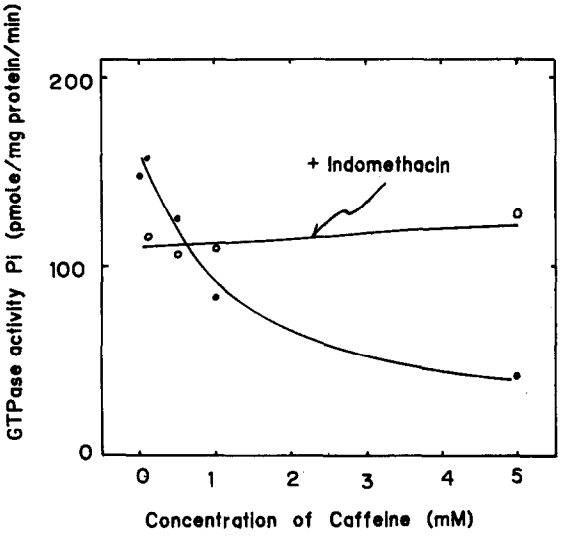

Fig.3. Effect of caffeine on GTPase activity; indo, 10 μM indomethacin, see fig.2.

Indomethacin reversed the inhibitory effects of caffeine and IP_3 on GTPase activity, suggesting that indomethacin inhibited the effect of caffeine or IP_3 on Ca^{2+} release from SR via a G-protein. Furthermore, the stimulatory effect of forskolin suggests that cAMP exerts an effect on this G-protein.

Perhaps the answer to the question as to why IP_3 and/or caffeine can increase the Ca^{2+} permeability

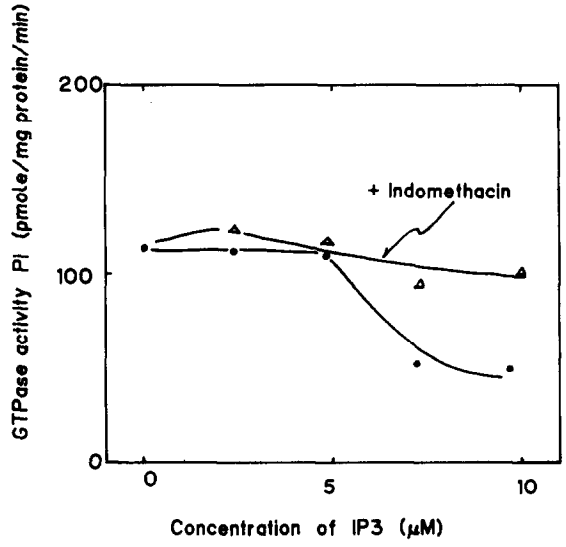

Fig.4. Effect of IP_3; indo, 10 μM indomethacin, see fig.2.

of the SR membrane is that IP_3 and/or caffeine stimulates the G-protein system of SR membranes and consequently can open the Ca^{2+} channel.

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