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L-Methionine Enhances the Contractile Responses of Rat Uterine Smooth Muscle to Acetylcholine and High KCl

Seiji ICHIDA
Department of Biological Chemistry, Faculty of Pharmacy, Kinki University, Higashi-Osaka 577, Japan
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Abstract—The contractile responses of isolated uterine smooth muscle from estradiol-treated ovariectomized rats to acetylcholine and high KCl in Ca-depleted modified Locke-Ringer (without CaCl₂ and with 0.1 mM EGTA) solution on addition of CaCl₂ (final concentration, 5 mM) are used as an indicator of Ca influx through the Ca channel. L-Methionine significantly enhanced these responses in the absence and presence of 3-deazaadenosine plus homocysteine thiolactone, blockers of methylation. These findings suggest that methylations of some components in isolated uterine smooth muscle seem to be important in stimulation-contraction coupling in the muscle.

The physiological significance of the protein- or phosphatidylethanolamine (PE)-methylation system has been demonstrated in various types of living cells (1-3). For example, the methylation of side chains such as carboxyl groups in proteins of cell membranes alters the surface charge and affects the structure and function of the cell membranes (4). Moreover, methylation of PE in cell membranes can increase membrane fluidity and affect coupling of beta-adrenergic receptors and adenylate cyclase in rat reticulocyte ghosts (2) and Ca²⁺ influx prior to histamine release in rat mast cells (2).

Recently, we reported that the contractile response of isolated uterine smooth muscle to acetylcholine (ACh) and high KCl in Ca-depleted modified Locke-Ringer (Ca-depleted Ringer) solution on addition of CaCl₂ resulted from increased influx of Ca ions into the muscle cells from the medium, and we suggested that this system was useful for studies on the mechanism(s) of voltage- and receptor-induced Ca influx (5).

In this work, I examined the effects of L-methionine (L-Met) and/or 3-deazaadenosine (3-DAA) plus homocysteine thiolactone (HCTL), which are blockers of methylation (1, 6), on the contractile responses of the muscle to ACh and high KCl in Ca-depleted Ringer solution on addition of CaCl₂ (final concentration, 5 mM).

Uterine smooth muscle of 17β-estradiol 3-benzoate (estradiol)-treated ovariectomized rats was prepared as described previously (5, 7-9). Virgin female Wistar rats weighing 150-200 g were used. On days 10 to 15 after ovariectomy, they were given 10 µg of estradiol once every 12 hr for 48 hr by i.s. injection and then sacrificed. Uterine horns were then isolated from estradiol-treated ovariectomized rats. The uterine horns were divided longitudinally into several segments (about 1.5 x 12 mm) which were each placed in a one-ml organ bath containing Ca-depleted Ringer solution with the following ionic composition (in mM): NaCl, 135.6; KCl, 5.4; ethylene glycol bis (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA), 0.1; MgCl₂, 0.2; KH₂PO₄, 0.2; NaHCO₃, 20; Na₂HPO₄, 0.6; and D-glucose, 2.8. The isotonic muscle tension was adjusted to about 100 mg. The contractile responses of the muscle to ACh and high KCl in Ca-depleted Ringer solution on addition of CaCl₂ (5 mM) were measured according to the previously described method (5). Briefly, uterine smooth muscle was immersed in Ca-depleted Ringer solution for a minimum of 60 min, kept at 30°C and bubbled con-
tinuously with 95% O₂+5% CO₂. For measurement of the contractile response to ACh, ACh was added to the muscle bath 2 min before addition of CaCl₂ (5 mM), while for measurement of the response to high KCl, the muscle was immersed in high KCl Ringer solution 10 min before addition of CaCl₂ (5 mM). The effects of L-Met and/or 3-DAA plus HCTL were examined by adding these compounds to the muscle bath 15 and/or 10 min, respectively, before addition of CaCl₂. As a control, the same volume of Ca-depleted Ringer solution was added instead of L-Met and/or 3-DAA plus HCTL at 15 and 10 min, respectively, before addition of CaCl₂ (5 mM). The contractile responses of the control were measured three to five times until the standard error of means was less than two percent.

Statistical analysis were carried out by Student’s t-test, and differences giving P<0.05 were considered as significant.

Estradiol was purchased from Teikoku Hormone Mfg. Co. (Osaka, Japan). HCTL and L-Met were from Sigma Chemical Co. (St. Louis, MO). 3-DAA was from Southern Research Institute (Birmingham, AL; from Dr. J.A. Montgomery). All drugs were prepared in Ca-depleted Ringer solution on the day of use and were neutralized when necessary.

As shown in Table 1, 3 mM L-Met in the absence and presence of 100 μM 3-DAA plus 100 μM HCTL significantly enhanced the contractile responses of isolated rat uterine smooth muscle to 3×10⁻⁶–3×10⁻⁴ M ACh or 30–60 mM KCl in Ca-depleted Ringer solution on addition of CaCl₂ (5 mM). The effect of L-Met depended both upon the time of treatment with L-Met and upon dose of L-Met (data not shown; Ichida et al., submitted for publication). 3-DAA plus HCTL significantly inhibited the contractile responses of the muscle to ACh and high KCl (Table 1), and its inhibitory effect was dose-dependent (data not shown; Ichida et al., submitted for publication). We also observed that protein carboxyl-methyltransferase and phospholipid methyltransferase are present in isolated uterine smooth muscle from estradiol-treated ovariectomized rats (Ichida et al., submitted for publication).

The physiological significance of the protein- or PE-methylation system has been demonstrated in various types of living cells

Table 1. Effects of L-Met and/or 3-DAA plus HCTL on the contractile responses to ACh and high KCl

<table>
<thead>
<tr>
<th>Conditions</th>
<th>ACh 3×10⁻⁴ M</th>
<th>KCl 30 mM</th>
<th>KCl 35 mM</th>
<th>KCl 40 mM</th>
<th>KCl 60 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.0±1.9 (8)</td>
<td>19.1±2.4 (12)</td>
<td>77.2±1.7 (10)</td>
<td>97.1±0.5 (10)</td>
<td>94.2±0.9 (10)</td>
</tr>
<tr>
<td>3 mM L-Met</td>
<td>34.0±2.4 (8)</td>
<td>36.4±6.1** (12)</td>
<td>83.5±2.3* (10)</td>
<td>99.0±1.1** (10)</td>
<td>99.3±1.8* (10)</td>
</tr>
<tr>
<td>100 μM 3-DAA</td>
<td>–</td>
<td>74.2±2.9 (16)</td>
<td>–</td>
<td>11.8±2.3 (16)</td>
<td>23.9±4.8 (16)</td>
</tr>
<tr>
<td>100 μM HCTL</td>
<td>–</td>
<td>40.8±3.3tt (12)</td>
<td>–</td>
<td>13.7±1.8t (9)</td>
<td>35.0±4.4tt (9)</td>
</tr>
<tr>
<td>3 mM L-Met</td>
<td>25.7±2.9 (9)</td>
<td>11.8±2.3 (16)</td>
<td>35.0±4.4tt (9)</td>
<td>65.8±5.6tt (9)</td>
<td></td>
</tr>
</tbody>
</table>

Uterine smooth muscle was immersed in Ca-depleted Ringer solution for a minimum of 60 min, kept at 30°C and gassed continuously with 95% O₂+5% CO₂. For measurement of the contractile response to ACh, ACh was added to the muscle bath 2 min before addition of CaCl₂, while for that to high KCl, the muscle was immersed in high KCl Ringer solution 10 min before addition of CaCl₂. The effects of L-Met and/or 3-DAA plus HCTL were examined by adding these compounds to the muscle bath 15 and/or 10 min, respectively, before addition of CaCl₂. Values are percentages (mean±S.E.) of the maximal response in the controls, for the number of animals shown in parenthesis. *P<0.05, **P<0.02, significance of difference from the control. tP<0.05, ttP<0.005, significance of difference from 3-DAA plus HCTL.
Therefore, my present findings suggest that S-adenosylmethionine-dependent methylations of protein and/or PE in isolated uterine smooth muscle may be involved in the contractile responses to ACh and high KCI in Ca-depleted Ringer solution on addition of CaCl₂ (5 mM). As discussed elsewhere (Ichida et al., submitted for publication), the enhancing effect of L-Met on these contractile responses can be explained by supposing that L-Met causes some change in the steps in stimulation-contraction coupling of rat uterine smooth muscle. However, further experiments are needed to clarify the exact step(s) in stimulation-contraction coupling in rat uterine smooth muscle at which L-Met is effective.

Recently, Sastry et al. (10) have reported that L-Met increased the contraction height of rat hemidiaphragm upon electrical stimulation of the nerve or the muscle, and they suggested that phospholipid methylation played a significant role in the functional activity of the rat diaphragm. We also found that in rat vas deferens, L-Met enhanced the contractile response to norepinephrine in Ca-depleted Krebs-Henseleit (without CaCl₂ and with 0.1 mM EGTA) solution on addition of CaCl₂ (5 mM) (Ichida et al., manuscript in preparation). From their findings and mine, the methylation of protein and/or phospholipid seems to be important in stimulation-contraction coupling in both skeletal (rat diaphragm) and smooth muscle (rat uterus and vas deferens).

References