L-Methionine Enhances the Contractile Response to Norepinephrine of Rat Vas Deferens

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Abstract—Under Ca-depleted conditions, the contractile responses of rat vas deferens in the presence of norepinephrine were not elicited until the addition of CaCl₂. L-Methionine enhanced the contractile response of vas deferens in the presence of methylation blockers under these conditions. The enhancing effect of L-methionine on some other smooth muscles could not be determined because under Ca-depleted conditions, these muscles showed 60–80% of the maximal contractile response on addition of CaCl₂ alone. These findings suggested that L-methionine has an enhancing effect on contraction of the rat vas deferens as it does on rat uterine muscle.

We have reported that when isolated uterine muscle from estradiol-treated ovariec-tomized rats was preincubated in Ca-depleted modified Locke-Ringer solution for a minimum of 60 min, the contractile responses of the muscle in the presence of acetylcholine (ACh), high KCl and serotonin were not elicited until addition of CaCl₂ (1), and have indicated that the contractile responses of the muscle under these conditions resulted from increased influx of Ca²⁺ ions into the smooth muscle cells from the medium (1). We also pointed out that this system is useful for studying the biochemical mechanism(s) of Ca²⁺ channels in the membrane.

Recently, we found that L-methionine enhances the contractile responses of isolated rat uterine muscle to ACh and high KCl in Ca-depleted modified Locke-Ringer solution on addition of CaCl₂, in the absence and presence of blockers of S-adenosyl-L-methionine-dependent transmethylation [3-deaza adenosine (3-DAA) plus homocysteine thiolactone (HCTL): (2–6)] (7, 8). We suggested that the enhancing effect of L-methionine may be partly due to S-adenosyl-L-methionine-dependent methylation of proteins and/or phospholipid in isolated uterine muscle.

In this work, we examined whether this enhancing effect of L-methionine could be observed in other isolated muscles besides rat uterine muscle, and we found that L-methionine also enhanced the contractile response of isolated vas deferens to norepinephrine (NE) in Ca-depleted Krebs-Henseleit (Ca-depleted K. H.) solution on addition of CaCl₂ (5 mM), in the presence of 3-DAA plus HCTL. A preliminary report of this work in abstract form has appeared (9).

Male and female Wistar rats weighing 150–250 g and male and female guinea-pigs weighing 250–300 g were used.

The ileum, stomach fundus, taenia coli and vas deferens of rats and guinea-pigs were isolated, and each was cut into several segments (about 1.5×12 mm). Each segment was placed in a one-ml organ bath containing Ca-depleted K. H. solution consisting of (in mM): NaCl, 122.0; KCl, 5.5; ethylene glycol bis-(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA), 0.1; MgCl₂, 1.6; NaH₂PO₄, 1.2; NaHCO₃, 20.0 and D-glucose 11.0. The medium was bubbled continuously with 5% CO₂ in O₂ (pH 7.4 at 36°C). The muscle preparations were equilibrated with this solution for a minimum of 60 min, and then
their contractile responses were examined.

The contractile responses of the various smooth muscles examined were measured isotonically, and the isotonic muscle tensions of rat ileum, stomach fundus and vas deferens, and guinea-pig ileum and taenia coli were adjusted to about 0.2, 1, 0.1, 0.2 and 1 g, respectively.

First, we examined whether contractile responses of segments of the various smooth muscles as described above in Ca-depleted K. H. solution could be induced by adding CaCl₂ (5 mM) alone, because contractile responses of segments of various smooth muscles in Ca-depleted K. H. solution to an agonist were induced by addition of CaCl₂ (5 mM). As seen in Table 1, on addition of CaCl₂ only, isolated segments of rat ileum, stomach fundus, guinea-pig ileum and taenia coli in Ca-depleted K. H. solution showed 68.6, 59.7, 84.9 and 67.8%, respectively, of their maximal contractile responses induced by ACh (at 3×10⁻⁴-3×10⁻² M, depending on the smooth muscle) plus CaCl₂ (5 mM). Therefore, in these tissues, we were unable to determine whether L-methionine enhanced the contractile response to ACh in Ca-depleted K. H. solution induced by adding CaCl₂ (5 mM). On the other hand, the contractile response of isolated rat vas deferens in Ca-depleted conditions induced by adding CaCl₂ (5 mM) alone was only 0.1% of the maximal contractile response to NE (3×10⁻⁴ M) plus CaCl₂ (5 mM). Therefore, we could examine the enhancing effect of L-methionine on contraction of isolated rat vas deferens.

The contractile response of isolated rat vas deferens to NE in Ca-depleted K. H. solution was measured as follows: After an equilibration period in Ca-depleted K. H. solution, NE (3×10⁻⁵-3×10⁻⁴ M) was added to the organ bath, followed 15 min later by CaCl₂ (5 mM). The effects of L-methionine and/or 3-DAA plus HCTL on the contractile responses to 3×10⁻⁵ and 3×10⁻⁴ M NE in Ca-depleted K. H. solution were examined by adding these compounds to the organ bath 15 and/or 10 min, respectively, before addition of CaCl₂ (5 mM). As a control, the same volume of Ca-depleted K. H. solution was added instead of L-methionine and/or 3-DAA plus HCTL. The contractile response of the control was measured three to six times until the range of variation of mean values was less than 5 percent.

EGTA, HCTL, L-methionine and NE were from Sigma Chemical Co. or Wako Co. 3-DAA was from Southern Research Institute. All drugs were prepared in Ca-depleted K. H. solution on the day of use and were neutralized when necessary.

Figure 1 shows the enhancing effect of L-methionine on the contractile responses of rat vas deferens to 3×10⁻⁵ and 3×10⁻⁴ M NE.

Table 1. Contractile responses of various smooth muscles under Ca-depleted conditions on addition of CaCl₂ (5 mM) alone

| Muscle          | Contractile response (of maximum) |
|-----------------|-----------------------------------|
| Rat             |                                   |
| ileum           | 68.6±6.4 (5)                      |
| stomach fundus  | 59.7±1.6 (10)                     |
| uterus†         | 1.5±0.4 (123)                     |
| vas deferens    | 0.1±0.1 (5)                       |
| Guinea-pig      |                                   |
| ileum           | 84.9±2.1 (5)                      |
| taenia coli     | 67.8±1.3 (5)                      |

Various smooth muscles were equilibrated in Ca-depleted K. H. or Ca-depleted modified Locke-Ringer solution for a minimum of 60 min. Then their contractile responses induced by adding CaCl₂ (5 mM) alone were measured. Values are means±S.E.'s for the numbers of animals shown in parentheses. 

*The maximal responses of various smooth muscles equilibrated in Ca-depleted conditions were measured by additions of NE (final conc. 3×10⁻⁴ M, for vas deferens) or ACh (final conc. 3×10⁻⁴-3×10⁻² M, for other muscles, the effective conc. depending on the kind of smooth muscle) plus CaCl₂ (5 mM). 

Data were cited from Reference 8.
Fig. 1. Effects of L-methionine and/or 3-DAA plus HCTL on the contractile responses of rat vas deferens to $3 \times 10^{-5}$ and $3 \times 10^{-4}$ M NE in Ca-depleted conditions on addition of CaCl$_2$ (5 mM). Columns and bars are means and S.E.'s for 8 to 14 animals. *P<0.02; **P<0.005: significance of difference from the value with 3-DAA plus HCTL.

in Ca-depleted K. H. solution on addition of CaCl$_2$ (5 mM) in the presence of 3-DAA plus HCTL. The contractile responses to $3 \times 10^{-5}$ and $3 \times 10^{-4}$ M NE (concentrations which produced half-maximal and maximal contractile responses, respectively) on addition of CaCl$_2$ (5 mM) were inhibited dose-dependently by 3-DAA plus HCTL. The inhibitory effects of 100 $\mu$M 3-DAA plus 100 $\mu$M HCTL on the contractile responses to $3 \times 10^{-5}$ and $3 \times 10^{-4}$ M NE were significantly attenuated in the presence of 3 mM L-methionine. In the presence of 30 $\mu$M 3-DAA plus 30 $\mu$M HCTL, the enhancing effect of L-methionine was not significant (P<0.05), but was reproducible in all experiments.

The contractile response of rat vas deferens to NE in Ca-depleted K. H. solution on addition of CaCl$_2$ was linked to the $\alpha_1$-adrenergic receptor (data not shown), and the response resulted from increased influx of Ca$^{2+}$ ion into the muscle cells from the medium, because the response was inhibited by Ca antagonists such as diltiazem, nitrendipine and verapamil (data not shown).

This work showed that L-methionine enhanced the contractile response of rat vas deferens to NE in Ca-depleted K. H. solution on addition of CaCl$_2$ in the presence of 3-DAA plus HCTL (Fig. 1). This effect of L-methionine on the contractile response of rat vas deferens was comparable with that on rat uterine smooth muscle reported previously (7, 8). However, we were unable to examine the enhancing effect of L-methionine alone on the contractile response of rat vas deferens to NE under the Ca-depleted condition, because the range of variation of mean values for the contractile response of control preparations to NE was greater with rat vas deferens than with rat uterine muscle, and because the enhancing effect of L-methionine alone on the contractile response of vas deferens seemed to be very small.

In this work, we used concentrations of $3 \times 10^{-5}$ and $3 \times 10^{-4}$ M NE in measurements of the contractile response of isolated rat vas deferens in Ca-depleted K. H. solution on addition of CaCl$_2$. The concentration of NE for half-maximal contraction was $2.9 \times 10^{-6}$ M (S. Ichida et al., unpublished data). These
concentrations of NE were higher than its physiological concentrations. The reason why such high concentrations were necessary is unknown, but may have been because we used rat vas deferens equilibrated with Ca-depleted K. H. solution for a minimum of 60 min.

The mechanism by which L-methionine enhances the contractile response of rat uterus or vas deferens to ACh or NE under Ca-depleted conditions on addition of CaCl2 is unknown. Possibly the enhancing effect of preincubation with L-methionine results from some change(s) in a step(s) of stimulation-contraction coupling of uterine muscle, as described previously (8). Sastry et al. (10) reported that L-methionine increased the contraction height of rat hemidiaphragm upon electrical stimulation of the nerve or the muscle. Moreover, recently, Landon et al. (11) reported that L-methionine enhanced the contractile response of rat aorta to high KCl. From their findings and ours, methylation(s) of protein and/or phospholipid seems to be important in stimulation-contraction coupling in both skeletal muscle and smooth muscles.

In summary, this work showed that L-methionine enhanced the contraction of rat vas deferens to NE under Ca-depleted conditions on addition of CaCl2. Its effect was similar to that on rat uterine muscle observed previously.

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