Hypothermia Enhances Acetylcholine-Induced Contraction of Isolated Rat Ileum

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Summary The amplitude of acetylcholine (ACh)-induced contraction of isolated rat ileum was enhanced at medium temperatures lower than normal body temperature. Maximum enhancement was achieved between 30 and 25°C. Changes in medium pH and activities of the enteric nervous system due to temperature changes were not essential for this enhancement.

Key words: hypothermia, ACh-induced contraction, ileum.

The effect of lowering temperature below normal body temperature on acetylcholine (ACh)-induced contraction of isolated rat ileum was examined in order to grossly investigate how cholinergic receptors and subcellular mechanisms controlling muscle contraction behave at a lower temperature range.

Experiments were performed from November 1985 to October 1986. Male albino rats, weighing 300–500 g, were used. Animals were stunned and bled. Longitudinal preparation of the ileum in 15-mm lengths was then dissected quickly, and clamped vertically in a Magnus tube of 25-ml capacity which was held in a thermal bath. Isometric contraction of the ileum preparation was recorded by means of a force-displacement transducer (TB-611T, Nihon Kohden, Japan) and carrier amplifier (AP-620G, Nihon Kohden). As a medium, conventional Tyrode solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 13.1 mM NaHCO₃, 5.56 mM glucose, 0.42 mM NaH₂PO₄) or modified Tyrode solution with 5 mM Tris-HCl buffer instead of phosphate and bicarbonate buffer, was used. Solution was continuously bubbled with a gas mixture of 95% O₂ and 5% CO₂.

Contraction of the preparation with 10⁻⁶M ACh (acetylcholine chloride, Sigma) was first induced at normal body temperature level, 37.0±1.0°C, and then at 34.0±1.0, 30.0±1.0, 25.0±1.0, and 20.0±1.0°C successively, and finally at 37.0±1.0°C again to ascertain the recovery from the cooling effect. Preparation was adapted to each temperature level at least 20 min before ACh was applied.

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Figure 1A shows the maximum amplitude measured by tension at the plateau phase of contraction of the ileum at different temperature levels in conventional Tyrode solution. Actual tension represented as 100% was 0.65 ± 0.09 g (mean ± S.E., n = 8). It is obvious that the amplitude of contraction reveals a bell-shaped enhancement at a temperature lower than normal body temperature. Maximum enhancement occurred between 30 and 25°C. Figure 1B shows rising time of muscle tension up to 70% of the maximum. The data to show recovery from cooling effect at 37°C is shown at the far right of the figure. Mean ± S.E. from 8 animals. Actual tension represented as 100% was 0.65 ± 0.09 g. Concentration of ACh was $10^{-6}$ M. Statistical significance between two data was examined with Student’s paired t-test (*p <0.05, **p <0.01).

Figure 1A shows the maximum amplitude measured by tension at the plateau phase of contraction of the ileum at different temperature levels in conventional Tyrode solution. Actual tension represented as 100% in the figure was 0.65 ± 0.09 g (mean ± S.E., n = 8). It is obvious that the amplitude of contraction reveals a bell-shaped enhancement at a temperature lower than normal body temperature. Maximum enhancement occurred between 30 and 25°C. Figure 1B shows rising time of muscle tension up to 70% of the maximum. The rising time of muscle
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Changes in temperature also affect pH of the medium. Conventional Tyrode solution, whose pH was set at 7.2–7.3 at 20°C after 10 min bubbling of a gas mixture, increased its pH by approx. 0.2 when there was a 20°C increase in temperature, i.e., pH 7.4–7.5 at 40°C. This change in pH might affect the properties of ACh-induced contraction of the ileum. To ascertain this possibility, we used modified Tyrode solution with Tris-HCl buffer. This solution changes its pH in an opposite way to the conventional solution. Tyrode solution with Tris-HCl, adjusted at pH 7.3–7.4 at 20°C after bubbling of a gas mixture, decreased its pH by 0.15 as temperature increased by 20°C, i.e., pH 7.25–7.15 at 40°C. This tendency of pH change resembles the change in pH of blood in a thermal gradient (ROSENTHAL, 1948). In this Tyrode solution with Tris-HCl buffer, the bell-shaped enhancement of ACh-induced contraction with the maximum between 30 and 25°C was also observed: 127, 155, 174, and 164% increase in amplitude at 34, 30, 25, and 20°C respectively (100% = 1.14 ± 0.16 g, n = 4). Enhancement of ACh-induced contraction was thus confirmed in two different buffer systems whose pH changed inversely in a thermal gradient. Therefore, we conclude that changes in pH of the medium are not essential for this enhancement of contraction. It is also reported that pH changes from 6.35 to 7.75 affected neither spontaneous nor ACh-induced contraction of the human intestine (HAYASHI et al., 1986).

Figure 2 shows that bell-shaped enhancement at a temperature range lower
than normal body temperature was still induced after the preparation was treated with $10^{-7}$ M tetrodotoxin (TTX, Sigma). TTX-treatment itself already enhanced the contraction with ACh even at 37°C. This is possibly due to an elimination of tonic inhibition of the muscle contraction by the enteric nervous system as mentioned by a previous work (Wood, 1972). Since, TTX suppresses the sodium channel activity, this result shows that changes in properties of the enteric nervous system by lowering temperature do not participate in the enhancement of contractile amplitude of the ileum. Further, $10^{-7}$ M atropine (atropine methylbromide, Sigma) completely abolished the ACh-induced contraction at all temperature levels examined. These results indicate that lowering temperature alters properties of muscarinic receptor and/or subcellular mechanisms responsible for muscle contraction.

Properties of ionic channels operated by muscarinic receptor are, however, scarcely the cause of the bell-shaped enhancement of ACh-induced contraction of the rat ileum in a temperature range lower than normal body temperature. Membrane potential of the guinea-pig urinary bladder muscle is most deeply hyperpolarized at 32°C, and depolarized in a linear fashion as temperature increases and decreases from this temperature (Kurihara et al., 1974). The expected effect of this depolarization by temperature changes is to increase sodium and calcium inward currents as reported in the guinea-pig taenia coli (Inomata and Kao, 1976) as a result of voltage sensitivity of muscarinic receptor-operated channels (Bolton, 1981). However, deeper hyperpolarization at temperatures where we have observed maximum enhancement of contraction does not provide any feasible possibilities that muscarinic receptor-operated channels are responsible for the bell-shaped enhancement of contraction.

Rapid cooling also causes contraction of the smooth muscle (Magaribuchi et al., 1973) presumably due to a release of intracellular calcium (Kurihara et al., 1974) as is the case for the skeletal muscle (Sakai et al., 1970). It is, therefore, possible that release and/or mobilization of intracellular calcium is potentiated by not only rapid but also moderate cooling as we have employed in the present experiments, and subserves the enhancement of ACh-induced contraction, although it is still insufficient to explain the reason why maximum enhancement occurs between 30 and 25°C.

The present results have demonstrated that ACh-induced contraction of the rat ileum is enhanced in a temperature range lower than normal body temperature due to intracellular mechanisms which follows an excitation of muscarinic receptors. This change in contractility of the ileum may be functionally significant in a hypothermic state: a decrease in temperature by only 2–3°C from the normal body temperature already caused enhancement of contraction (Figs. 1 and 2). The precise mechanism causing bell-shaped enhancement of contraction will be further analyzed.

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