The Effects of Acetylcholine on the Electrical and Mechanical Activity of the Smooth Muscle of the Rabbit's Mesenteric Vein

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Electrical activities have been recorded from single smooth muscle cells in the blood vessels of frog (1, 2), turtle (3), rat (4, 5), guinea pig (6, 7, 8) and rabbit (9). In the present experiments, simultaneous records were made of electrical and mechanical activities of isolated muscle strips from rabbit anterior mesenteric veins in order to study the effects of acetylcholine on them.

Longitudinal muscle strips, 3 mm wide and 10 mm long, were cut from the isolated anterior mesenteric veins after the connective tissue and the thick layer of adventitia surrounding the vessel had been carefully removed under binocular microscope. These strips showed conspicuous spontaneous phasic contractions when they were suspended in Kreb's Ringer solution at 36°C to 37.5°C.
The normal Kreb's Ringer solution used in the experiments contained (mM) : NaCl 120.7, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.5, NaH_2PO_4 1.2, Glucose 11.5 and was aerated with 95 % O_2 + 5 % CO_2.

The transmembrane potential of the single smooth muscle cell was measured with floating microelectrode, with high resistance (30-70MΩ). mounted on a micromanipulator. The tip potential of microelectrodes varied between 5 and 20mV. Muscle tension was measured by means of strain gauge transducer (Nihon Kohden)

In all preparations studied here could be seen action potentials and phasic contraction (Fig. 3). Action potential occured singly or in succession (Fig. 1). Sometimes slow potential alone was recorded without spike potential (Fig. 1-b).

Generally in the smooth muscle of the mesenteric vein of rabbit, like that of peripheral blood vessel of the frog (1, 2), and mesenteric artery of the guinea-pig (6), but unlike that of mammalian viscera (10, 11, 12), a definite correlation between spike discharge and phasic contraction was not observed (Fig. 3-a). The observation may be explained by the poorer conduction and, therefore, the asynchronization of the activity in different cells.

Acetylcholine produced strong contractions of mesenteric vein (Fig. 2). The threshold concentration was about 10^{-8} g/ml (Fig. 2-a) and the maximum response was obtained with about 10^{-6} g/ml (Fig. 2-b), but the muscle gradually developed tachyphylaxis to acetylcholine (Fig. 2). Acetylcholine-induced tension

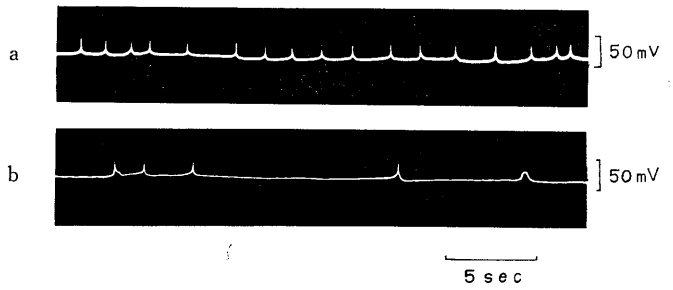

Fig. 1. Spike activity recorded intracellulary from the mesenteric veins.

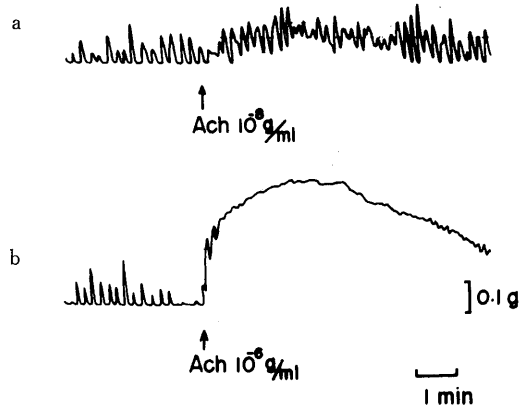

Fig. 2. Mechanical responses of a mesenteric vein to acetylcholine (a; 10^{-8} g/ml, b; 10^{-6} g/ml)

development was blocked by atropine (Fig. 3), suggesting this action being mainly muscarinic. The frequency of action potential was increased conspicuously with acetylcholine (10^{-6} g/ml) and atropine blocked this effect, thus decreasing the frequency or abolishing the action potential, without appreciable change in the membrane potential (Fig. 3). Therefore, the contraction of mesenteric vein by acetylcholine and the blocking effects of atropine against it seemed to depend only on the change in frequency of action potential. However acetylcholine caused additional contraction of the vein immersed in K-rich solution in which the strips had been depolarized and probably could have shown no significant electrical change to any drug (Fig. 4). Hence it appeared that acetylcholine-induced contraction of mesenteric vein depended not only on increase in spike activity of the smooth muscle membrane but also on the other mechanism. A possible explanation for the mechanism seemed to be related to the essential role of the Ca ions in the contraction of the vascular smooth muscle (13).

It has already been established that the development of tension in cardiac (14) and skeletal muscles (15, 16) is determined by the concentration of the available Ca ions within the cell. If the same holds for vascular smooth muscles, it is conceivable that acetylcholine may bring about contraction by increasing the concentration of available Ca ions within the cell by unknown mechanism.

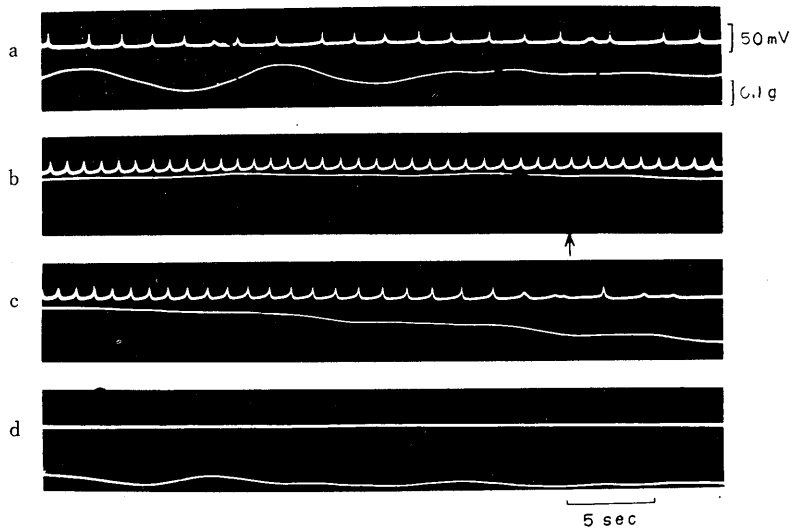

Fig. 3. The excitatory effect of acetylcholine (10^{-6} g/ml) and the blocking effect of atropine (10^{-5} g/ml) to acetylcholine (10^{-6} g/ml) on a mesenteric vein.
a; Control
b; 5 minutes after acetylcholine (10^{-6} g/ml)
b, c and d; Continuous records. Atropine (10^{-5} g/ml) was applied at arrow. Upper traces electrical, lower mechanical.

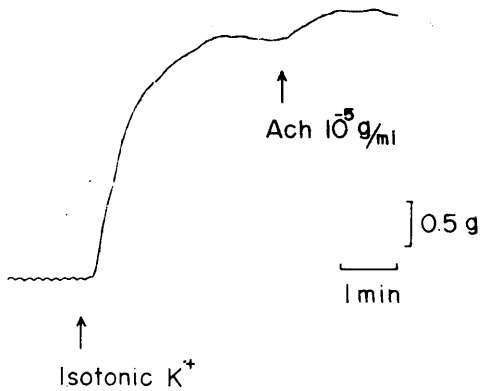

Fig. 4. Mechanical response of a mesenteric vein in potassium-rich solution to acetylcholine.

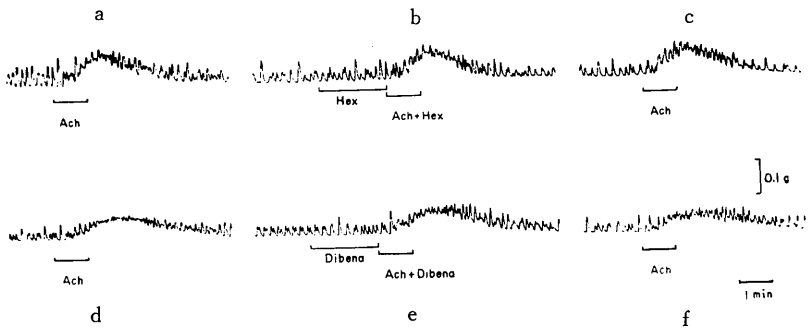

Fig. 5. The effects of hexamethonium (upper row) and dibenamine (lower row) on mechanical responses to acetylcholine.
a, b ; Control responses to acetylcholine (Ach 10^{-7} g/ml)
b, e ; Two minutes after administration of hexamethonium (Hex 5×10^{-5} g/ml) or dibenamine (Dibena 10^{-5} g/ml) acetylcholine (Ach 10^{-7} g/ml) was applied.
c, f ; The responses to acetylcholine (Ach 10^{-7} g/ml) after washing with Kreb's solution.

Noradrenaline as well as acetylcholine increased electrical and mechanical activities of mesenteric vein (17). This result proposed the possibility that acetylcholine did not act directly on the muscarinic receptors but produced excitable effects by causing the release of noradrenaline from the peripheral storage sites of postganglionic nerve (18) or by secondarily raising the excitability of adrenergic postganglionic nerve fiber following stimulation of preganglionic nerve.

From this point of view, Holman tried to establish a model of the innervation of the mesenteric vein of the sheep (19). Her experimental results showed that high concentration of hexamethonium (5×10^{-5} g/ml) blocked the excitatory effect of acetylcholine, though insufficiently. On the basis of this fact, she suggested the existence of the above mentioned mechanisms in the mesenteric vein preparations. In our experiment, however, hexamethonium (5×10^{-5} g/ml) did not block the excitatory effect of acetylcholine (Fig. 5). Moreover α-receptor blocking agents, e.g. dibenzyline 10^{-5} g/ml and dibenamine 10^{-5} g/ml, also did not block the effect (Fig. 5). At present it is not clear why the different results from ours were obtained by her.

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