EFFECT OF BIOLOGICALLY ACTIVE SUBSTANCES (ACETYLCHOLINE, HISTAMINE, AND BRADYKININ) ON DEPOLARIZED SMOOTH MUSCLE OF THE TAENIA COLI

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Experiments on isolated strips of the guinea pig taenia coil showed that compound D-600, which blocks calcium channels, depresses the contractile response of depolarized smooth muscle to administration of acetylcholine ($5 \cdot 10^{-6}$), histamine ($2 \cdot 10^{-6}$), and bradykinin (10^{-5} g/ml). Besides depressing the effect of biologically active substances, compound D-600 also depresses the "potassium contracture." It is postulated that the biologically active substances activate the flow of Ca^{++} ions through the surface membrane but not the discharge of Ca^{++} from the sarcoplasmic reticulum.

KEY WORDS: smooth muscles; potassium depolarization; biologically active substances; calcium channels.

The following scheme to explain the action of biologically active substances on smooth muscle under normal conditions is now generally accepted: interaction between the biologically active substance and chemosensitive areas of the membrane–increased permeability of these areas to ions (possibly Na^+ or Ca^{++}) carrying the inward current–membrane depolarization–development of the action potential (or increased firing rate in the case of a spontaneously active cell)–increase in the Ca^{++} flow through slow Na–Ca-channels–increased Ca^{++} concentration in the myoplasm–development of contraction. However, it is known that histamine, acetylcholine (AC) and noradrenalin can evoke contractions in muscles immersed in isotonic potassium solution [1, 4, 7]. In that case the membrane is completely depolarized and the slow Na–Ca channel inactivated. The question naturally arises, on account of what is the concentration of calcium (an activator of contractions, see [10]) in the myoplasm increased? There are three possible sources of Ca^{++}: extracellular Ca^{++}, Ca^{++} stored in the membrane, and Ca^{++} stored in the sarcoplasmic reticulum (SPR).

The object of this investigation was to study the level at which biologically active substances exert their action on depolarized smooth muscle. Is it limited to the surface membrane or do the biologically active substances induce the liberation of Ca^{++} directly from SPR? To answer this question the compound D-600 (a methoxy derivative of verapamil), a specific blocker of Ca channels, was used.

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips (1.5-2 cm long, 500-600 μ thick) of guinea pig taenia coli. The potential was recorded by the "sucrose gap" technique [9]. Stretching of the muscle was measured under isometric conditions by means of a mechanotron. The following solutions were used (in mM): normal Krebs' solution – NaCl 120.7; KCl 5.9; NaH_2PO_4 1.2; $NaHCO_3$ 15.5; $MgCl_2$ 1.2; $CaCl_2$ 2.5; glucose 11.5; "potassium solution" – KCl 167.7; $KHCO_3$ 3.6; $CaCl_2$ 0.4; glucose 11.5. The temperature in all experiments was 25 ± 1°C. The solutions were oxygenated with a mixture of 96% O_2 and 45% CO_2. The pH of all solutions was 7.3.

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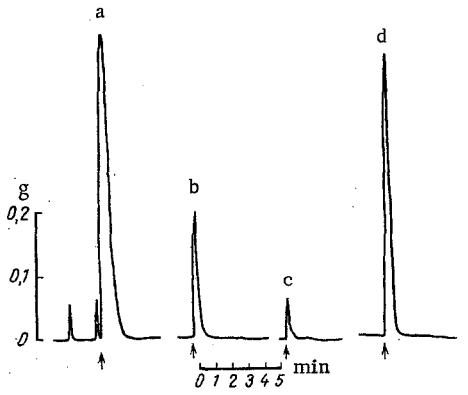

Fig. 1

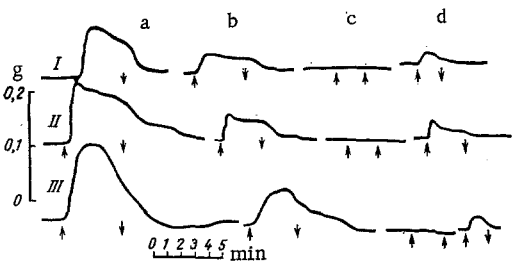

Fig. 2

Fig. 1. Effect of D-600 on "potassium contracture" in smooth muscle of guinea pig taenia coli: a) potassium contracture; b) potassium contracture after preliminary treatment of the preparation for 10 min with compound D-600; c) potassium contracture after treatment for 30 min with D-600; d) potassium contracture after rinsing D-600 out of the preparation with normal Krebs' solution containing 6.6 mM $CaCl_2$.

Fig. 2. Effect of compound D-600 on contractile response to biologically active substances – histamine ($2 \cdot 10^{-6}$), AC ($5 \cdot 10^{-6}$), and bradykinin (10^{-5} g/ml) in depolarized smooth muscle of guinea pig taenia coli. Ia, IIa, IIIa) Effects of AC, histamine, and bradykinin, respectively in depolarized smooth muscle of taenia coli; Ib, IIb, IIIb) the same after treatment with D-600 for 10 min; Ic, IIc, IIIc) the same after treatment with D-600 for 30 min; Id, IId, IIId) partial recovery of effect after rinsing for 20 min with potassium solution containing 4.4 mM $CaCl_2$. Arrow pointing upward) addition of biologically active substance; arrow pointing downward) rinsing preparation to remove substance. Ordinate, contraction of muscle, in g.

EXPERIMENTAL RESULTS AND DISCUSSION

In all the experiments the strips of taenia coli in normal Krebs' solution possessed spontaneous electrical and mechanical activity. On replacement of the normal solution by potassium solution, lasting depolarization of the membrane developed, accompanied by strong contracture (Fig. 1a). The magnitude of the subsequent spontaneous relaxation of the muscle in hyperpotassium solution depended on temperature: the higher the temperature, the higher the level of residual contraction [4]. The temperature in these experiments was so chosen that contraction of the muscle returned to its initial level. Preliminary perfusion of the muscle (5 experiments) with normal Krebs' solution containing D-600 (10^{-6} g/ml) for 20-30 min caused the contracture in response to the change of solution hardly to develop at all (Fig. 1c).

Addition of biologically active substances – histamine ($2 \cdot 10^{-6}$ g/ml), AC ($5 \cdot 10^{-6}$ g/ml), and bradykinin (10^{-5} g/ml) – to the potassium solution caused contraction (Fig. 2), the magnitude and duration of which depended both on the substance used and on its concentration. It will be clear from Fig. 2 that in response to addition of the substance to the solution mechanical contraction developed, then spontaneously diminished despite the continuous addition of the substance. On rinsing the preparation with potassium solution the contraction returned to its original level and in some experiments the muscle tone after rinsing became less than initially. Further addition of the substance gave virtually the same effect.

Perfusion of the muscle with potassium solution containing D-600 (10^{-6} g/ml) for 20-25 min before addition of the biologically active substances reduced their effect (Fig. 2c). Slow development of the blocking effect and its irreversibility are special features of the action of compound D-600. The partial restoration of the effect of the biologically active substances was obtained by prolonged (20-30 min) rinsing of the preparation with potassium solution with an increased Ca^{++} concentration (4.4-6.6 mM). The restorative action of the increased Ca^{++} concentration was evidently due to competition between Ca^{++} and D-600 for the same sites of the membrane [6, 8].

Let us consider two alternative schemes of the action of biologically active substances leading to contraction of depolarized smooth muscle: 1) The substance acts on the membrane, the Ca channels are opened, and Ca^{++} enters the cell along the concentration gradient; 2) the substance penetrates into the cell, acts directly on SPR, and activates the liberation of Ca^{++}. The first scheme does not exclude the participa-

tion of SPR in the act of contraction; Ca^{++} ions, having passed through the membrane into the cells, can trigger (regeneratively) the discharge of Ca^{++} from SPR [3, 5]. The fact that compound D-600 abolishes the effect of biologically active substances prevents either of these suggested schemes from being chosen. If the biologically active substance acts only on the surface membrane, activating the inward flow of Ca^{++} through it, compound D-600, by blocking the Ca channels, ought to abolish the effect of the substance. If, however, AC, histamine, and bradykinin act directly on SPR, in that case D-600 should abolish the effects of the substances, for blocking the Ca channels in the membrane may (although this is not necessary) lead to deprivation of SPR of its calcium. On rinsing the preparation with potassium solution with an increased Ca^{++} concentration, the muscle tone and, consequently, the Ca^{++} concentration in the myoplasm and SPR are restored. In that case, restoration of the effect of the biologically active substances would be expected; however, experiments show that the effects are virtually not restored. Consequently, the only possible explanation is that the biologically active substances act on the cell membrane.

The cell membrane of the taenia coli is practically not in contact with SPR, which accounts for about 2% of the cell volume [2]. In smooth muscles with a more highly developed SPR (5% of the total volume or more) some of its vesicles form direct contact with the membrane [2]. In that case, the biologically active substances could act on a common site of the surface membrane and SPR membrane. This could explain the fact that D-600 (or verapamil) does not completely abolish the effect of biologically active substances in the smooth muscle of the aorta [1] and large arteries [2]. Besides abolishing the effect of biologically active substances, D-600 depresses the "potassium contracture" arising on replacement of the normal solution by the potassium solution. This also indicates that the flow of Ca^{++} through the surface membrane is the decisive factor in the act of contraction.

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