COMPARATIVE ANALYSIS OF THE ACTION OF PAPAVERINE, ISOPRENALINE, La^{3+}, AND COMPOUND D-600 ON DEPOLARIZED GUINEA PIG TAENIA COLI SMOOTH MUSCLE

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Experiments on strips of guinea pig taenia coli were carried out by the double sucrose gap method. Isometric contractions of the strip in the region of the artificial node were recorded during perfusion with a solution containing a high concentration of potassium (120 μM KCl, 47mM NaCl), to which one of the myolytics and agents inducing contraction of depolarized muscle were successively added. "Off-responses" (OR) arising after the end of stimulation by square hyperpolarizing pulses (11 sec) also were studied. D-600 (10^{-5} g/ml) and La^{3+} ($2 \cdot 10^{-4}$ g/ml) were shown to reduce muscle tone (residual potassium contracture) and to abolish the contractile effects of histamine or of a tenfold increase in the Ca^{2+} concentration in the medium $[Ca]_0$. The amplitude of OR fell but its kinetics remained unchanged. Papaverine (10^{-5} g/ml) caused a lasting decrease in tone, abolished the effects of an increase in $[Ca]_0$ or addition of histamine, but caused virtually no change in the amplitude of OR – the only effect was a sharp increase in the rate of muscle relaxation after the end of its contraction phase. Isoprenaline (10^{-6} g/ml) lowered the tone of the depolarized muscle without changing the contractile effects of histamine, an increase in $[Ca]_0$ or OR. The results point to differences in the mechanisms of action of the various myolytics. D-600 and La^{3+} block chemically excitable and all types (fast, slow, and uninactivated) of electrically excitable Ca channels. The action of isoprenaline is evidently connected with activation of the system of cyclic AMP synthesis and, consequently, with increased sequestration of Ca^{2+} in the intracellular depots. To understand the nature of the action of papaverine, it has to be suggested that it can block chemically excitable Ca channels and also electrically excitable Ca channels that have been open for a long time.

KEY WORDS: papaverine; isoprenaline; La^{3+}; compound D-600.

This investigation is a continuation of previous studies of the mechanisms of the spasmolytic action of certain chemical agents on smooth muscle depolarized by K^+ ions. Its aim was to compare the effects of La^{3+} and compound D-600, direct blockers of calcium channels, with the action of the classical spasmolytics papaverine and isoprenaline (isopropylnoradenalin). The effects of these agents was judged from changes in: 1) tone of depolarized muscle, 2) the amplitude and time course of "off-responses" (OR) consisting of contractions of the muscle on discontinuation of a long-acting hyperpolarizing current, 3) the effects of application of histamine to the depolarized muscle, and 4) the effects of increasing the Ca^{2+} concentration in the medium $[Ca]_0$.

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips of guinea pig taenia coli (1.5–2 cm long and 300–500 μ thick). The double sucrose gap method was used to stimulate the muscle and record the membrane potential simultaneously. The design of the experimental chamber was described previously [3]. The following solutions were used (in mM): a) 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; b) potassium depolarizing solution: KCl 120, NaCl 47.7, $NaHCO_3$ 3.6, $CaCl_2$ 0.4, glucose 11.5. Papaverine, isoprenaline, and D-600 were added directly to the potassium solution. $LaCl_3$ was added to potassium solution in which the $NaHCO_3$ was replaced by Tris (5.8 mM). The pH of all solutions was 7.3. All experiments were carried out at room temperature and the solutions were not oxygenated (an explana-

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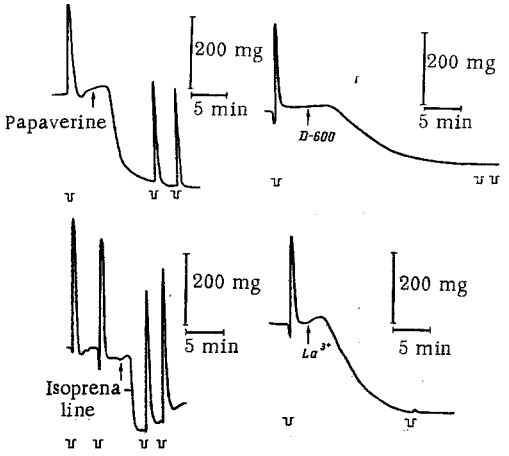

Fig. 1

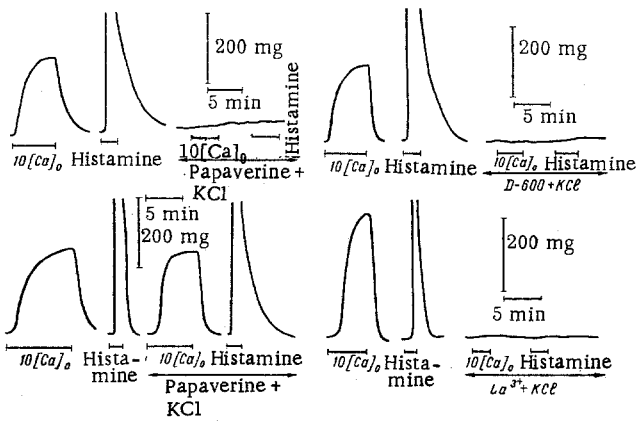

Fig. 2

Fig. 1. Effect of papaverine (10^{-5} g/ml), isoprenaline (10^{-6} g/ml), D-600 (10^{-5} g/ml), and $LaCl_3$ ($2 \cdot 10^{-4}$ g/ml) on tone and OR of depolarized muscle. Times of application of hyperpolarizing current (square pulses) indicated below; all records obtained on different preparations.

Fig. 2. Effects of tenfold increase in $[Ca]_0$ and application of histamine (10^{-5} g/ml) in normal depolarizing solution and after previous action of papaverine (10^{-5} g/ml), isoprenaline (10^{-6} g/ml), D-600 (10^{-5} g/ml), and $LaCl_3$ ($2 \cdot 10^{-4}$ g/ml). Horizontal lines indicate periods of action of drugs.

tion of these conditions was given by Pogadaev and Timin [2]). The order of the experiments was as follows: The muscle was first placed in Krebs' solution and contracted automatically; if no automatic contractions took place, contractions were induced by application of short pulses of depolarizing current. The Krebs' solution was then replaced by depolarizing solution. After the end of the phasic part of potassium contracture and establishment of a constant level of muscle tone, 11-sec pulses of hyperpolarizing current were applied to the muscle. An OR of the muscle developed to discontinuation of this current. Contraction of the muscle was then induced by application of histamine (10^{-5} g/ml) to it or by a tenfold increase in $[Ca]_0$ in the external solution. Application of the spasmolytic to the depolarized muscle caused a decrease in tone, and when the tone was established at a constant level the muscle was tested for its ability to give an OR and to contract in response to application of histamine or a solution with increased $[Ca]_0$.

EXPERIMENTAL RESULTS

In papaverine, D-600, and $LaCl_3$ the tone of the depolarized muscle fell and then maintained at a constant level, whereas in isoprenaline, after an initial fall, the tone gradually increased (Fig. 1). Application of papaverine, after the preliminary action of isoprenaline, led to a further sharp decrease in muscle tone (not shown in Fig. 1). The amplitude of OR in papaverine and isoprenaline on average remained unchanged compared with normal. During application of D-600 or La^{3+} a sharp decrease in tone and total disappearance or OR were observed.

The effects of a tenfold increase in $[Ca]_0$ and histamine are shown in Fig. 2. Against the background of papaverine, D-600, and La^{3+} the responses of the muscle to an increase in $[Ca]_0$ and to application of histamine disappeared, whereas in isoprenaline they remained the same as in normal depolarizing solution. It was stated above that D-600 and La^{3+}, in concentrations of $1 \cdot 10^{-5}$ and $2 \cdot 10^{-4}$ g/ml respectively, completely inhibited OR; for that reason, in order to study the action of these agents on the descending phase of OR, the concentrations of D-600 and La^{3+} were reduced to $1 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ g/ml respectively. Under these conditions the drop in amplitude of OR took place slowly, so that changes in their descending phase could be examined. Graphs of the descending phase of OR, plotted on a semilogarithmic scale, are shown in Fig. 3 (counting began from the time of maximal contraction and each curve was normalized with respect to the maximum of amplitude). Graphs of the descending phase of OR in normal depolarizing solution and of the descending phase of automatic contractions in Krebs' solution are shown for comparison. Clearly papaverine strongly accelerated the descending phase of OR and its velocity approximated to that of relaxation in Krebs' solution. In isoprenaline, D-600, and La^{3+} the velocity of the descending phase of OR was virtually not increased despite a considerable reduction in initial muscle tone.

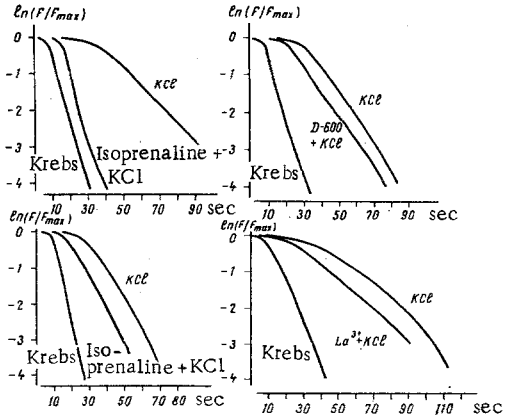

Fig. 3. Descending phases of OR plotted on semilogarithmic scale during action of papaverine (10^{-5} g/ml), isoprenaline (10^{-6} g/ml), D-600 (10^{-6} g/ml), and $LaCl_3$ (10^{-5} g/ml). Abscissa, time (in sec); ordinate, ratio ln F/F_{max}), where F is the force developed by the muscle and F_{max} the amplitude of the given contraction.

A common feature of the action of the various agents used was a decrease in tone of the depolarized muscle. It follows from the fact that La^{3+} and D-600, direct blockers of calcium channels, cause a decrease in tone of the depolarized muscle and that this tone is maintained by the steady-state Ca^{2+} flow along certain uninactivated calcium channels that are open in the depolarized membrane. These same channels are evidently responsible for the contraction effects during an increase in $[Ca]_0$. La^{3+} and D-600 are evidently universal blockers of calcium channels, since they depress both the steady-state Ca^{2+} flow through the depolarized membrane and transient Ca^{2+} flows leading to action potential generation, OR formation, and the contraction effects of biologically active substances [2, 3, 5].

The spasmolytic action of papaverine and isoprenaline on the normally polarized muscle is generally connected with the fact that they increase the intracellular cyclic AMP concentration [4, 6]. Under the influence of papaverine this may take place as a result of inhibition of phosphodiesterase, which hydrolyzes cyclic AMP, whereas when isoprenaline is used, it may take place through activation of adenylate cyclase, responsible for cyclic AMP synthesis. Elevation of the cyclic AMP level in the cell activates the sequestration of Ca^{2+} in the intracellular depots and thereby promotes relaxation of the muscle [4].

However, the results of these experiments indicate an essential difference in the action of papaverine and isoprenaline on depolarized smooth muscles. The fact that isoprenaline blocks neither the effects of histamine or the contractile responses to a tenfold increase in $[Ca]_0$ nor OR suggests that its action is unconnected with blocking of the calcium channels. Most probably relaxation of the depolarized muscle in isoprenaline is actually due to stimulation of cyclic AMP synthesis with consequent activation of Ca^{2+} elimination from the myoplasm into the intracellular depots and the external solution. By contrast, an essential role in the action of papaverine on depolarized smooth muscle is played by blocking of the inflow of Ca^{2+} into the cell [1]. Evidence of this is given by the fact that papaverine inhibits the effect of a tenfold increase in $[Ca]_0$ and the contractle effect of histamine. It can tentatively be suggested that papaverine blocks the Ca^{2+} flow along channels fixed in the open state in depolarized muscle, and along chemically excitable calcium channels activated by histamine. Meanwhile, papaverine has no appreciable effect on electrically excitable calcium channels responsible for action potential generation and for OR [1]. A very characteristic feature in the effect of papaverine is acceleration of the descending phase of OR while the amplitude of this contraction remains virtually unchanged. The writers postulated previously [1] that the descending phase of OR is determined both by liberation of Ca^{2+} from the myoplasm and by slow inactivation of electrically excitable calcium channels. In the light of this hypothesis, the narrowing of OR in papaverine can be explained on the grounds that it blocks only those calcium channels which are open for a sufficiently long time; papaverine does not act, however, on fast calcium channels, which mainly determine the amplitude of OR. The absence of any narrowing of OR in D-600 and La^{3+} indicates that these agents evidently block all types of calcium channels in smooth muscle to an equal degree.

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EFFECT OF LEUCINE-ENKEPHALIN ON INTERNEURONAL TRANSMISSION OF EXCITATION

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Experiments on unanesthetized cats immobilized with flaxedil showed that injection of leucine-enkephalin (1 mg) into the lateral ventricle is followed by inhibition of evoked responses in the ventrolateral columns of the spinal cord and of segmental interneuronal transmission in the spinal cord and by a decrease in the amplitude of potentials in cortical somatosensory area I evoked by sciatic nerve stimulation. Naloxone (1 mg/kg, intravenously) prevented these effects of leucine-enkephalin. Preliminary injection of methysergide (2.5 mg/kg, intraperitoneally) led to weakening of the effect of leucine-enkephalin on spinal interneuronal transmission. Leucine-enkephalin did not change the amplitude of polysynaptic potentials of the glossomandibular reflex, the arc of which is closed in the brain stem.

KEY WORDS: leucine-enkephalin; naloxone; interneuronal transmission of excitation; methysergide.

Narcotic analgesics are known to influence interneuronal transmission of excitation at different levels of the CNS [1-3]. Analgesia is produced by these substances through their specific interaction with opiate receptors, present in various regions of the CNS [13]. Serotoninergic mechanisms also take part in the development of the analgesic effect of drugs belonging to the morphine group [15]. The pentapeptides leucine-enkephalin and methionine-enkephalin are endogenous neuromediators with morphine-like activity [7, 11]. Electrical stimulation of the raphe nuclei induces analgesia [9], which is evidently connected with an increase in the liberation of polypeptides with morphine-like activity [6]. There is thus a definite parallel between the effects of narcotic analgesics and of morphine-like polypeptides.

The object of this investigation was to study the effect of one morphine-like polypeptide, namely leucine-enkephalin, on interneuronal transmission of excitation in the CNS, on the character of its interaction with naloxone, a specific antagonist of the narcotic analgesics, and with methysergide, which blocks serotoninergic receptors.

EXPERIMENTAL METHOD

Experiments were carried out on unanesthetized cats of both sexes, weighing 2.8-3.5 kg, immobilized with flaxedil (2 mg/kg, intravenously). The preliminary manipulations – dissection of the sciatic nerve, spinal cord, anterior roots of the cord at the level of segments L7-S1, trephining of the skull, and catheterization of veins and arteries – were carried out under ether anesthesia. Artificial ventillation was provided by the DP-5 apparatus at the rate of 205 ml air/kg body weight·min [8]. Evoked potentials, in response to stimulation of the central end of the divided sciatic nerve with single supramaximal (5-12 V) pulses of current 0.1 msec in duration, were recorded in cortical somatosensory area I on the contralateral side, and also in the ventrolateral columns of the spinal cord at the level of segments L2-L3 on both ipsi- and contralateral sides. Monosynaptic and polysynaptic responses in the anterior roots of the spinal cord during analogous sciatic nerve stimulation and polysynaptic potentials in the central structures of the glossomandibular reflex, arising in the

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