THEOPHYLLINE-INDUCED CHANGES IN THE PRESYNAPTIC EFFECTS OF ADENOSINE, BACLOFEN, CLONIDINE, AND MORPHINE IN THE GUINEA PIG MYENTERIC PLEXUS

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Activation of α_2-adrenoreceptors and of A_1-adenosine, opiate, and GABA receptors in spinal primary afferent terminals leads to inhibition of presynaptic transmitter release by means of a common mechanism, namely lowering of the intracellular cAMP concentration and inhibition of the function of voltage-dependent Ca channels [1]. However, it is not clear whether this mechanism is responsible for inhibition of presynaptic transmitter release in other (e.g., cholinergic) neurons.

The contractions of the guinea pig small intestine evoked by transmural stimulation and the accompanying acetylcholine release are inhibited by morphine [6], adenosine [4], sympathomimetics [9], GABA, and baclofen [5] as a result of their action on presynaptic receptors of myenteric plexus neurons of the intestine [4, 5, 8]. Whereas the presynaptic effects of the above-mentioned agonists in cholinergic neurons are also brought about by a fall in the intracellular cAMP concentration, they can be inhibited by theophylline, which raises the cAMP concentration in the cells by inhibiting phosphodiesterase. According to data in the literature [7] theophylline, by blocking adenosine receptors, competitively inhibits not only the presynaptic effect of adenosine, but also the analogous effect of morphine, although it is not a blocker of opiate receptors.

This paper gives data on the effect of theophylline on presynaptic inhibition of the function of cholinergic neurons of the myenteric plexus of the guinea pig small intestine caused by a number of agonists (adenosine, baclofen, clonidine, and morphine).

EXPERIMENTAL METHOD

Experiments were carried out on segments of the isolated small intestine from guinea pigs weighing 300-400 g. The intestinal segments were immersed in Krebs' solution aerated with oxygen at 36°C. Intestinal contractions were induced by transmural low-frequency (0.1 Hz) stimulation by square pulses 3 msec in duration, and with a voltage of 10-20 V, and recorded under isometric conditions by means of a strain-gauge transducer on a KSP-4 potentiometer. Dependence of the degree of inhibition of intestinal contractions, induced by transmural stimulation, on the concentration of the agonist was studied in the absence and presence of theophylline (10^{-5}-10^{-4} M) or of 1,3-dipropyl-8-phenylxanthine (DPPX; 10^{-8}-10^{-6} M). The action of each agonist was studied in 3-5 concentrations of 4-8 intestinal segments. Activity of the agonists was estimated as EC_{50}, and activity of the antagonists (theophylline and DPPX) as pA_2 and the degree of the maximal shift (unaccompanied by any change of slope) of the log of concentration versus effect of the agonist curves in the presence of the antagonist.

EXPERIMENTAL RESULTS

All the agonists studied reduced contractions of the small intestine evoked by low-frequency transmural stimulation. Clonidine exhibited high activity (EC_{50} = 26.0 ± 7.8 nM). Baclofen, on the other hand, was active only in millimolar concentrations (Table 1).

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TABLE 1. Presynaptic Activity of Agonists and of Theophylline as Their Antagonist (M ± m)

Agonist	Number of experiments	EC_{50} of agonist, M	Theophylline as antagonist	
			pA_2	maximal shift of curves to the right, order of magnitude
Adenosine	8	$(5,95\pm0,46)\cdot10^{-7}$	4,76±0,04	1,18
Baclofen	4	$(1,77\pm0,40)\cdot10^{-4}$	3,62±0,03	0,60
Clonidine	5	$(2,60\pm0,78)\cdot10^{-8}$	3,85±0,02	1,23
Morphine	4	$(1,07\pm0,43)\cdot10^{-7}$	3,46±0,03	0,28

Theophylline counteracted the presynaptic effects of adenosine, baclofen, and clonidine and had a rather weaker action on the effects of morphine (Table 1). In the presence of theophylline in concentrations of $3\cdot10^{-5}$–10^{-4} M a parallel shift of the curves of log of concentration versus effect of adenosine was observed, and the degree of the shift was proportional to the increase in theophylline concentration, so that the antagonism of theophylline with adenosine can be interpreted as competitive. These results agree with data in the literature [3, 7]. Conversely, theophylline showed noncompetitive antagonism with the other agonists. The effect of clonidine was counteracted by theophylline in concentrations of $3\cdot10^{-5}$ and 10^{-4} M to the same degree, but antagonism with baclofen and morphine was exhibited by theophylline only in a concentration of 10^{-4} M, and higher concentrations reduced the slope of the curves and the maximum of the effects. Values of pA_2 for theophylline as an antagonist of baclofen, clonidine, and morphine coincided, but differed roughly by an order of magnitude from pA_2 of theophylline as an antagonist of adenosine (Table 1).

Differences in the values of pA_2 are evidence that the antagonism of theophylline with clonidine, baclofen, and morphine takes place at the "trans-receptor" level and may be due to the inhibitory effect of theophylline on phosphodiesterase and to lowering of the cAMP level in the muscle cells. Evidence in support of this view is given by the fact that DPPX, which blocks adenosine receptors, differs from theophylline in not inhibiting phosphodiesterase [3], and exhibits a high degree of antagonism with adenosine (pA_2 = 7.29 ± 0.06; maximal shift 1.3 orders of magnitude), but in concentrations up to $3\cdot10^{-6}$ M it did not change the presynaptic effects of baclofen, clonidine, and morphine.

Presynaptic inhibition of the function of the cholinergic neurons of the myenteric plexus, induced by baclofen, clonidine, and morphine, is thus a cAMP-dependent phenomenon. The degree of presynaptic inhibition caused by these agonists is reduced by a theophylline-induced increase in the intracellular cAMP concentration which, according to data in the literature [2], is accompanied by stimulation of the function of voltage-dependent Ca-channels.

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