Reaction of Neurons to Alkaloid Agonists of Opioid Receptors during Modulation of Phosphodiesterases

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We studied the effect of ultralow doses of theophylline and morphine, modulators of receptors and intracellular signal systems, on ion permeability of membranes. Theophylline and morphine in ultralow doses blocked the neuronal responses to these agents applied in physiological concentrations. Theophylline in ultralow doses attenuated, but did not completely block neuronal reaction to morphine. These findings suggest that ultralow doses of various substances producing no appreciable physiological changes can activate mechanisms providing optimum response to strong factors.

Key Words: *ultralow doses; signal systems; ion permeability of membrane; theophylline; morphine*

Weak short-term exposures and ultralow doses of bioactive substances in stimulate the adaptive mechanisms at various levels, including the molecular level. Weak factors probably activate various mechanisms aimed at the formation of long-term adaptive reactions and trigger stereotypic intracellular programs. These changes provide optimal conditions for the reaction to strong stimuli. At the molecular and cell levels, adaptive reactions include changes in ion permeability (IP) of the plasma membrane. Changes in membrane permeability to Ca^{2+} and H^+ modulate intracellular calcium signaling system and protein activity. Variations in membrane permeability for Na^+ and K^+ maintain ion homeostasis and regulate cell excitability.

IP of membranes is controlled by intracellular signaling systems. Disturbances in IP accompany various pathological states, *e.g.* convulsive activity, encephalopathy, and ischemia [2]. In the present study an attempt was undertaken to modulate membrane IP by affecting intracellular signal systems with low doses of theophylline. Theophylline increased neuronal permeability for Ca^{2+}, which is probably related to the increase in intracellular cAMP concentration [3]. The concentration of cAMP in cell is regulated by phosphodiesterases (PDE) catalyzing conversion of cAMP into AMP (Fig. 1). It should be emphasized that PDE are involved (directly or indirectly) in the function of three signaling systems in cells. Theophylline inhibits PDE, which leads to elevation of neuronal cAMP content and increases permeability for Ca^{2+} (Fig. 1). Agonists of opioid receptors initiate cAMP synthesis [5]. We studied the effects of substances modulating intracellular signaling systems and administered in ultralow doses on IP of membranes. Experiments were performed with theophylline and alkaloid agonist of opioid receptors morphine that increase the influx of ions [3,4,6].

MATERIALS AND METHODS

Experiments were performed on cultured neurons of *Lymnaea stagnalis* (0.5-1.5 years) isolated enzymatically and placed in a special chamber for electrophysiological studies. The medium contained 85 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 1.5 mM $MgCl_2$, and 8 mM Tris-HCl (pH 7.6-7.8). Ionic currents were recorded using patch-clamp technique. Glass micropipettes were introduced to neurons. The cell was attracted to a pore (8-10 μ) with a low negative pressure (1-10 gPa). Voltage clamping and recording of integral ion currents were performed under the micropipette. These currents were approximately 100-500 pA. The experiment, data collection, and processing were performed using special equipment and software.

Morphine (10 mM) and theophylline (1 mM) in dilutions of 10^{-12} (C6), 10^{-60} (C30), and 10^{-100} (C50) prepared by homeopathic technology were delivered into incubation medium using a microdispenser and removed by a flow system.

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0007-4888/03/supp-0017$25.00

RESULTS

Experiments were performed on neurons demonstrating an increase in the amplitude of influx currents in response to morphine (about 12% cells, Fig. 2, *a*). The amplitude of ionic currents increased slowly and peaked 30-40 min after application of morphine. This effect of morphine was reversible: the shape and amplitude of ionic currents returned to initial values after 45-60-min washout with physiological saline in a flow system [1]. Further experiments with morphine were performed on neurons, when the amplitude of ionic currents returned to initial level.

Theophylline in physiological doses (1 mM) increased the amplitude of inward currents (by 30-80%, Fig. 2, *a*) and excitability of neurons 30-40 min after administration. These changes persisted for 1.5 h. The effect of theophylline was reversible and the shape and amplitude of ionic currents returned to normal after washout.

Theophylline in various dilutions (C6, C30, and C50) produced no significant changes in the amplitude of inward currents. The effects of C6 theophylline (n=20) were qualitatively similar to those produced by physiological concentrations. However, in these doses theophylline increased the amplitude of inward currents by 3-10% only in 40% cells. This effect was short-lasting and the amplitude of ionic currents returned to the initial level after 5-25 min.

Theophylline in dilutions of C30 and C50 had no effects on neuronal excitability and amplitude of inward currents (n=49). Minor changes (increase or decrease by 3-5%) did no affected the mean value, however, dispersion of the results increased (Fig. 2, *a*).

We studied the effects of consecutive treatment of neurons with test substances in physiological concentrations. Experiments were performed on cells with pronounced reaction to theophylline (increased amplitude of inward currents) after pretesting with morphine. Theophylline was removed by washing. When the amplitude of inward currents returned to the initial level, morphine was added in the experimental chamber. The neuronal response to morphine did not differ by direction, amplitude, and dynamics from changes

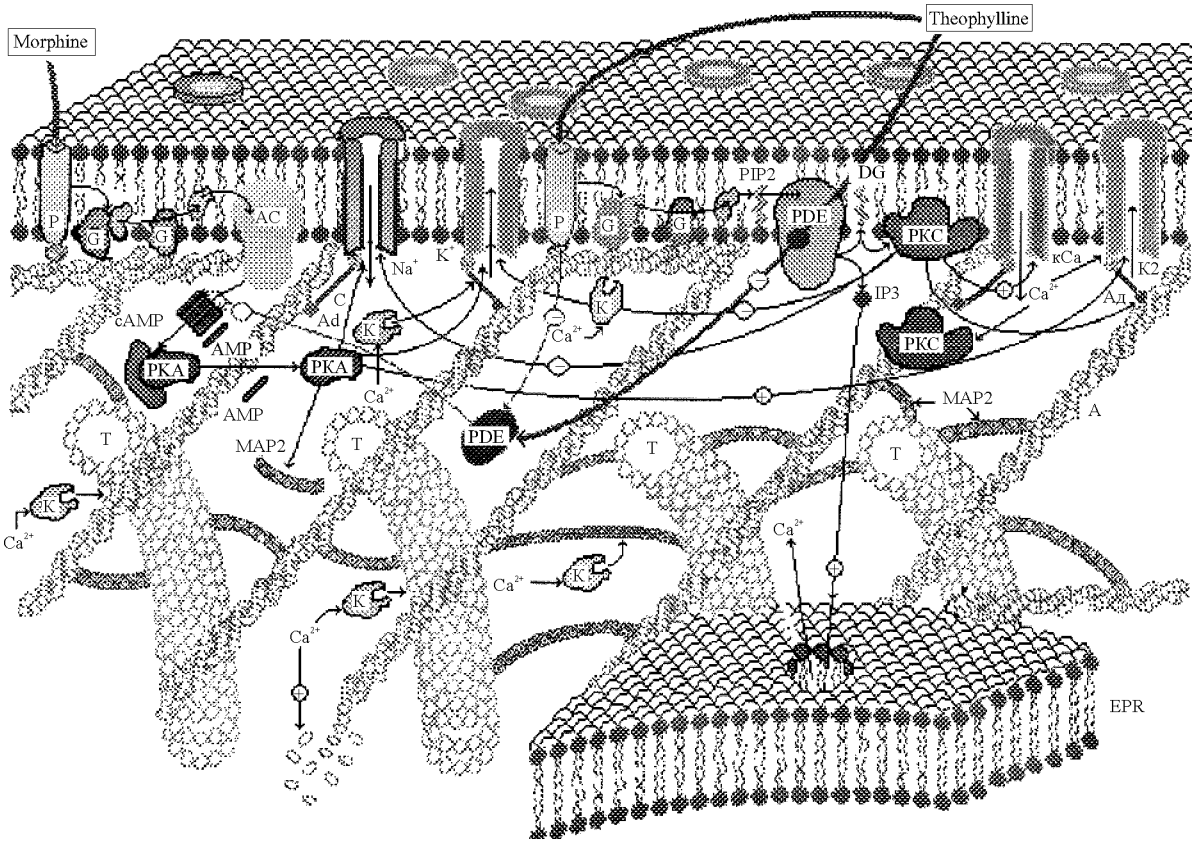

Fig. 1. Simplified scheme of interaction between second messengers and ionic channels during modulation of phosphodiesterase activities and reaction of neurons to alkaloid agonists. *R*, receptors; *G*, *G* proteins; *AC*, adenylate cyclase; *PKA*, protein kinase A; *PIP2*, phosphatidylinositol-4,5-diphosphate; *PDE*, phosphodiesterases; *DG*, diacylglycerol; *IP3*, inositol triphosphate; *PKC*, protein kinase C; *C*, calpain; *EPR*, endoplasmic reticulum; *T*, tubulin; *A*, actin; *MAP2*, microtubule-associated protein-2; *Ad*, addisin; *S*, spectrin; *Na*, sodium channel; *K*, potassium channel; *Ca*, calcium channel; *K2*, calcium-dependent potassium channel. "+", positive relationship; "—", negative relationship.

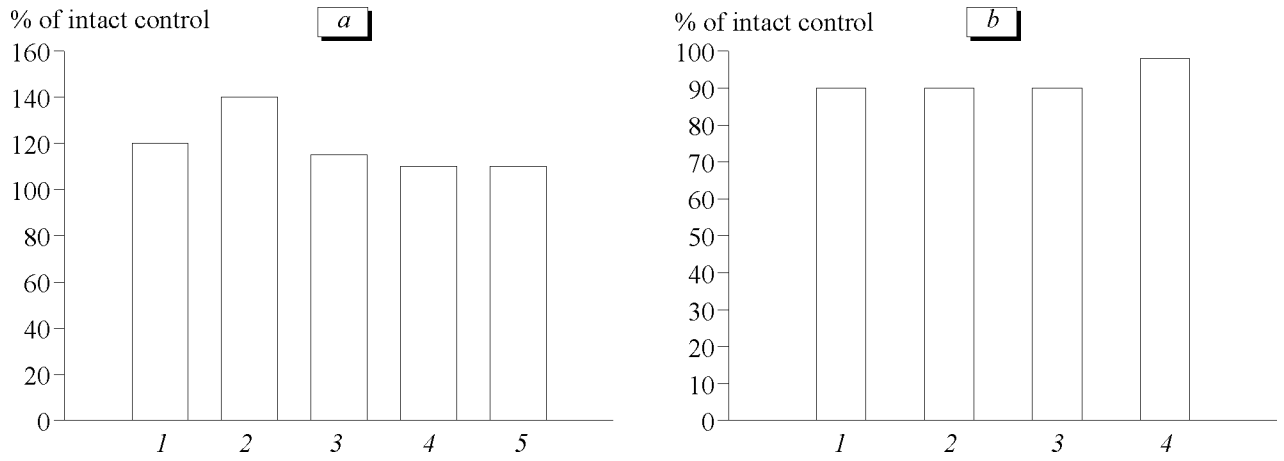

Fig. 2. Amplitude of transmembrane inward currents during individual (*a*) and successive administration (*b*) of theophylline and morphine in various concentrations. *a*) morphine in concentrations of 1 (*1*) and 10 mM (*2*); theophylline in dilutions of C6 (*3*), C30 (*4*), and C50 (*5*). *b*) Administration of morphine in concentrations of 1 (*1-3*) and 10 mM (*4*) after treatment with theophylline C6 (*1*), C30 (*2, 4*), and C50 (*3*).

observed in control cells during pretesting. We observed a long-lasting increase in the amplitude of inward currents and excitability of neurons. The effect was reversible. The amplitude of ionic currents returned to normal after washout.

For evaluation of neuronal reaction to successive treatment with ultralow and physiological doses of theophylline the neurons were incubated with ultralow concentration for 40 min and then theophylline in a concentration of 1 mM was added. Preincubation ultralow doses of theophylline abolished the increase in the amplitude of inward currents produced by its physiological concentrations (Fig. 2, *b*).

For evaluation of the effects of ultralow doses of theophylline and physiological doses of morphine neurons after pretesting with morphine (n=8) were incubated with theophylline C30 and then treated with morphine. Morphine modulated inward currents in all studied neurons. However, effects produced by morphine in intact neurons (pretest) and cells treated with theophylline C30 were different. After preincubation of neurons with theophylline C30 morphine increased the amplitude of inward currents by 5-15%. These changes developed 30-40 min after addition of morphine and persisted for 20-30 min (Fig. 2, *b*).

Thus, theophylline and morphine in ultralow doses blocked neuronal response to these agents in physiological concentrations [1]. Theophylline in ultralow doses attenuated, but did not completely block the reaction of neurons to morphine. Our results suggest that various substances in ultralow doses producing no appreciable physiological changes can activate the mechanisms providing optimum response to strong factors.

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