Role of Hepatic Opioid Receptors in the Regulation of Bile Excretion

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Experiments on isolated rat liver perfused with Ringer—Krebs bicarbonate buffer showed that stimulation of δ-opiate receptors in this organ increases bile flow rate and taurocholate secretion. Stimulation of μ-opiate receptors decelerated bile production and inhibited taurocholate secretion. Acceleration of bile production and stimulation of taurocholate secretion under the influence of dalargin is probably related to its interaction with δ-opiate receptors in the liver.

Key Words: *liver; opiate receptors; dalargin; DADLE; DAGO*

The system of endogenous opioid neuropeptides plays an important role in the regulation of the function of the gastrointestinal tract (GIT). Opioid peptides are widely distributed in the nervous system and GIT [7]. They protect the gastric mucosa [5], regulate secretory activity of the pancreas [10], modulate metabolic processes in the liver [1], and produce a hepatoprotective effect during acute liver damage [15].

The effects of opioid peptides on GIT are regulated by hierarchically different systems, including the central and autonomic nervous systems [6,9]. The liver contains δ- and μ-opiate receptors (OR) [14]. Opioid peptides affect secretion of bile acids in bile, which modulates bile flow [4]. The action of opioid peptides realized at a hierarchically higher level of regulation (nervous system) can mask the effects of OR in the organ [8].

Here we studied the role of liver OR in the regulation of bile excretion.

MATERIALS AND METHODS

Experiments were performed on the isolated liver from outbred albino rats. The liver was perfused with Ringer—Krebs bicarbonate buffer containing 100 μmol/liter sodium taurocholate at 37°C [11,13]. The perfusion solution was oxygenated with a mixture of 95% O_2 and 5% CO_2 at pH 7.2-7.4. Perfusion was performed via the hepatic artery (30%, 120 cmH_2O) and portal vein (70%, 20 cmH_2O) at a flow rate of 30 ml/min [12].

Bile was collected 10 min after the start of perfusion. Opioid peptide analogues were added to the perfusion solution immediately before collection of bile.

The rate of bile production was measured 30 min and 1 h after the start of bile collection using graduated micropipettes. The concentration of sodium taurocholate in bile was estimated spectrophotometrically in a modified Zlatkis—Zak reaction [2].

We used the following synthetic analogues of opioid peptides: δ- and μ-OR agonist, officinal lyophilized Dalargin (Tyr-D-Ala-Gly-Phe-D-Leu-Arg, 5 μg/liter perfusion solution); selective δ-OR agonist DADLE ([D-Ala^2,D-Leu^5]-enkephalin, VektorBioProdukt, 5 μg/liter perfusion solution); selective μ-OR agonist DAGO ([D-Ala^2,N-MePhe^4,Gly^5-ol]-enkephalin, VektorBioProdukt, 5 μg/liter perfusion solution); selective κ-OR agonist U50488H ([trans-(+/-)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]benzeneacetamide] hydrochloride, Up-

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John, 0.5 mg/liter perfusion solution); μ-OR antagonist naloxone hydrochloride (Solyaris, 0.5 mg/liter perfusion solution); and norbinaltorphimine (Sigma-Aldrich, 1 mg/liter perfusion solution). Naloxone hydrochloride was used in a dose that inhibited various types of OR.

The results were analyzed by Mann—Whitney test. The differences were significant at $p<0.05$.

RESULTS

The rate of bile secretion and intensity of cholate secretion in the isolated liver were much lower compared to intact liver, which is consistent with published data [13]. The rate of bile secretion increased 30 and 60 min after nonselective stimulation of δ- and μ-OR with dalargin (by 67.2 and 48.5%, respectively, $p<0.001$, Fig. 1). Taurocholate secretion increased 30 and 60 min after treatment (by 40.3 and 34.5%, respectively, $p<0.001$, Fig. 2).

The rate of bile secretion increased 30 and 60 min after selective stimulation of δ-OR in the liver with DADLE (by 63.9 and 56%, respectively, $p<0.001$, Fig. 2). Taurocholate secretion increased 30 and 60 min after treatment (by 48.5 and 37.4%, respectively, $p<0.001$, Fig. 2).

The rate of bile secretion decreased 30 and 60 min after administration of selective μ-OR agonist DAGO (by 54.1 and 44.0%, respectively, $p<0.001$, Fig. 1). Taurocholate secretion significantly decreased 30 and 60 min after treatment (by 61.45 and 60%, respectively, $p<0.001$, Fig. 2).

Addition of OR antagonist naloxone hydrochloride to the perfusion solution completely blocked the effects of dalargin, DADLE, and DAGO on secretion in the isolated liver (Figs. 1 and 2).

Addition of selective κ-OR antagonist U50488 to the perfusion solution had little effect on the rate of bile secretion and intensity of cholate secretion in the isolated liver (Figs. 1 and 2). These para-

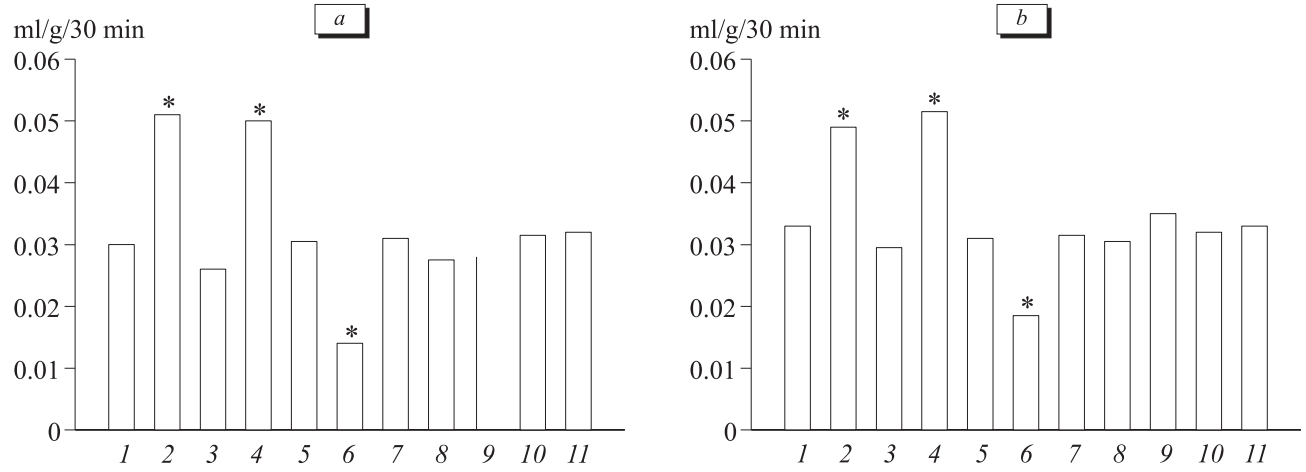

Fig. 1. Effect of synthetic analogues of opioid peptides on bile flow rate in isolated rat liver. Here and in Fig. 2: 30 min after the start of study (*a*); 60 min after the start of study (*b*). Control (perfusion with bicarbonate buffer, *1*); dalargin (*2*); dalargin+naloxone (*3*); DADLE (*4*); DADLE+naloxone (*5*); DAGO (*6*); DAGO+naloxone (*7*); U50488 (*8*); U50488+norbinaltorphimine (*9*); naloxone (*10*); norbinaltorphimine (*11*). *$p<0.001$ compared to the control group.

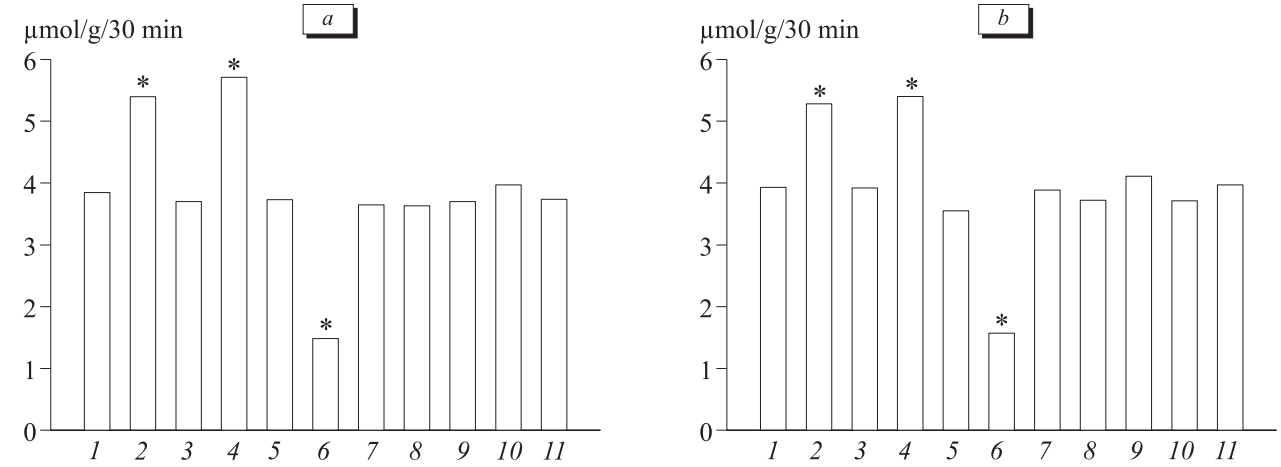

Fig. 2. Effect of synthetic analogues of opioid peptides on sodium taurocholate secretion in the isolated rat liver.

meters remained practically unchanged after treatment with κ-OR antagonist norbinaltorphimine.

These data indicate that selective stimulation of δ-OR in the liver with DADLE, as well as nonselective stimulation of δ- and μ-OR with dalargin, accelerated bile secretion and stimulated cholate secretion. Selective stimulation of μ-OR in the liver with DAGO inhibits secretory function of the isolated liver. The observed effect of dalargin is probably related to its affinity for δ-OR.

Our previous studies showed that intraperitoneal injection of selective μ-OR agonist DAGO increases bile flow rate in the intact liver of narcotized rats [3]. However, stimulation of μ-OR in the isolated liver has the opposite effect on bile secretion. Hence, some direct effects of opioid peptides on bile secretion in the whole organism can be masked by the higher levels of hierarchic regulation.

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