Vasoconstrictor Effect of Acetylcholine in Veins of the Liver

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Intraportal acetylcholine administered to narcotized rats produced atropine-resistant constriction of hepatic veins, which was considerably prevented by phentolamine. Sodium nitroprusside produced a vasodilator effect. Similar results were obtained on isolated venous strip from the portal vein: acetylcholine-induced contraction was reduced by 25-50% in the presence of nicotinic receptor antagonist tubocurarine and cholinergic agonist nicotine and by 10% in the presence of tetrodotoxin. Probably, acetylcholine stimulates synthesis and release of a vasoconstrictor transmitter via nicotinic receptors of endothelial cells and/or portal vascular wall nerve terminals.

Key Words: *acetylcholine; portal vein; nicotinic cholinergic receptors; endothelium; vasoconstriction*

Acetylcholine (Ach) and some other substances dilate arterial vessels via a special mediator (NO) produced by vascular endothelium [9]. It was hypothesized that Ach exerted similar action in veins by modulation of endothelial NO synthesis. Paradoxically, Ach and cholinergic agonists constrict liver veins. This effect was observed *in situ* on rats and *in vitro* on isolated strips of the portal and mesenteric veins [1,6,10,11]. Specifically, the constrictor response to stimulation of cholinergic nerve terminals or injection of Ach was observed in hepatic vein, posterior vena cava, and some other liver veins, and in pulmonary vessels [3]. Despite the data on the existence of vasoconstrictor reaction to Ach in various veins, its mechanism remains unknown.

Our aim was to clarify the mechanism of Ach-induced contraction of hepatic portal veins.

MATERIALS AND METHODS

Acute experiments were carried out on random-bred albino rats narcotized with urethane (1 g/kg) or nembutal (35 mg/kg). Blood pressure (BP) in the carotid artery and portal vein (PVP) was recorded with an EMT-31 electromanometer. An LP-9 polarograph was used to measure local blood flow in the liver by the method of hydrogen clearance, in which hydrogen was produced electrochemically. A modified RG 4-01 rheograph was employed to evaluate changes in blood filling of the liver. The above data were documented on an H 071.6M pen recorder. The test substances were Ach (0.8-1.2 mg/kg), norepinephrine (1-5 mg/kg), atropine (0.5-2.0 mg/kg), saponin (2 mg/kg), sodium nitroprusside (20-80 μg/kg), and phentolamine (0.5-1.0 mg/kg), which were injected intraportally.

In special experimental series, contractions of the isolated strip of the portal vein (PV) were recorded with a mechanotron 6MX1C, DC amplifier made on the basis of 140 ChD8 operational amplifier, C1-64A oscilloscope, and H338-4P pen ink recorder. The preparation was mounted in a plexiglass chamber and perfused with Tirode solution at 37.0±0.5°C. The test substances Ach (1×10^{-5}-5×10^{-5} M), nicotine (10^{-4} M), tubocurarine (1.5×10^{-6}-1.5×10^{-4} M), or tetrodotoxin (10^{-4} M) were introduced into bathing solution at the rate of 2.5 ml/min. The data were processed statistically using Student's *t* test.

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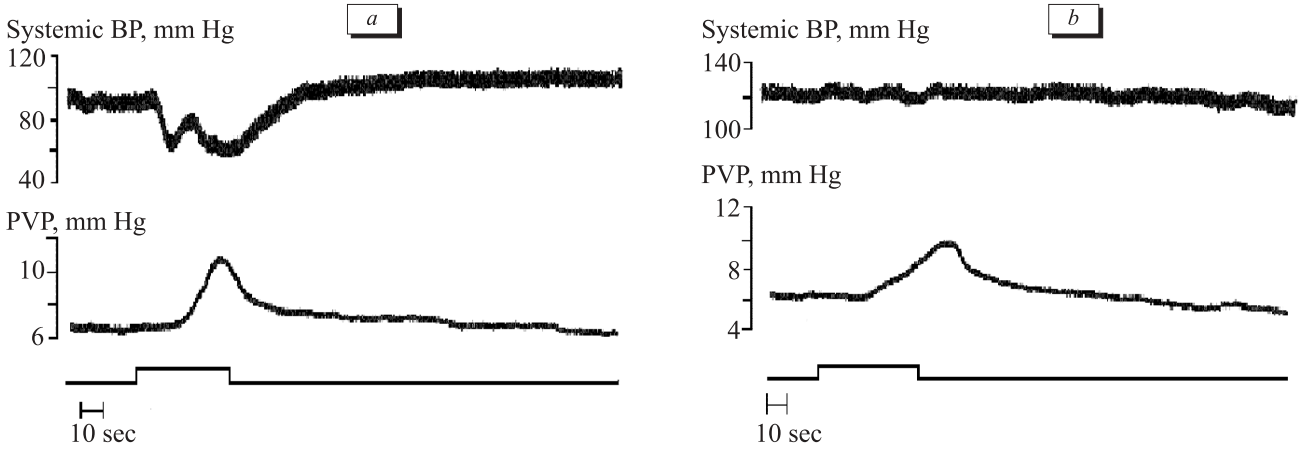

Fig. 1. Effect of intraportal Ach (1.2 μg/kg) on BP and PVP before (*a*) and during (*b*) blockade of nicotinic cholinergic receptors with atropine (0.5-1.0 mg/kg).

RESULTS

Our previous experiments on dogs confirmed the constrictor effect of Ach on PV. In this work further analysis of this phenomenon was carried out on rats with the following initial parameters: BP 85.4±5.7 mm Hg, PVP 6.3±0.3 mm Hg, local (hepatic) blood flow 99.3±2.5 ml/(min×100 g), and hepatic blood filling 23.3±2.4 ml/100 g. The intraportal injection of Ach to narcotized rats decreased BP by 32-39%, increased PVP by 25-44%, and decreased local blood flow and hepatic blood filling by 23-25% ($p<0.05$). These data unequivocally indicate constriction of intrahepatic and portal blood vessels. Atropine inhibited the depressor response of systemic BP, but produced no effect on the pressor response to Ach in PV (Fig. 1).

De-endothelization of hepatic veins by intraportal administration of saponin eliminated the pressor response of PV to Ach, but produced no significant effect on similar response of PV to norepinephrine, which was used to test the integrity of muscular layer in portal vessels. The response to norepinephrine was considerably (by 60-78%) prevented by phentolamine, which also decreased Ach-induced pressor responses of hepatic veins (to 5% of the initial value).

Therefore, in hepatic venous bed Ach produced its effect via the endothelium similarly as it does in the great majority of the arteries. However, in contrast to the arteries, Ach induced constriction of hepatic veins. This unusual response to Ach could be explained by functional peculiarities of smooth muscle cells in these vessels or the potency of endothelial cells in hepatic veins to produce some constrictor factors in response to Ach. Intraportal administration of sodium nitroprusside (NO donor) decreased BP by 48%, PVP by 17% ($p<0.001$), and increased blood filling of the liver by 18.2% ($p<0.01$), and decreased local blood flow by 23.1% ($p<0.05$), which attests to dilation of hepatic veins. The response to sodium nitroprusside was preserved after de-endothelization of hepatic venous bed. Therefore, NO produced by sodium nitroprusside dilate not only hepatic arteries, but also hepatic veins, while venous endothelium in the presence of Ach produces vasoconstrictor substances.

All the known subtypes of the muscarinic cholinoceptors are sensitive to atropine [4], so tolerance of Ach-induced response of hepatic veins to atropine (Fig. 2) implies that this reaction is mediated via nicotinic cholinoceptors. Such receptors were recently found in endothelial cells of blood vessels [7,8]. Probably, activation of these receptors with Ach in our experiments induced synthesis and release of some vasoconstrictor factors such as norepinephrine by the venous endothelial cells. This

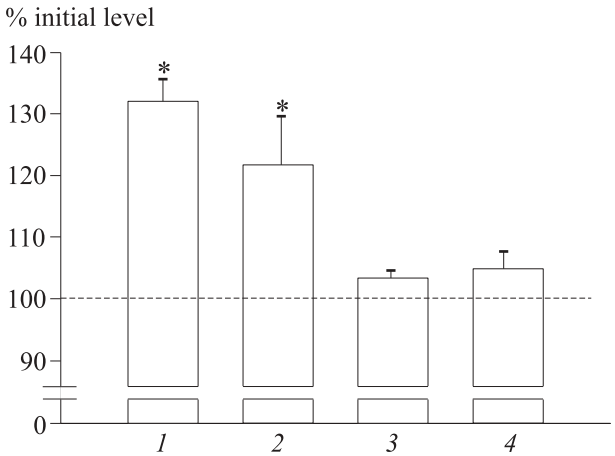

Fig. 2. Effect of Ach (1 μg/kg) on PVP in control (*1*), under the action of atropine (*2*), after de-endothelization (*3*), and during the action of phentolamine (*4*). Dash line marks the initial level. *$p<0.001$ compared to the initial level.

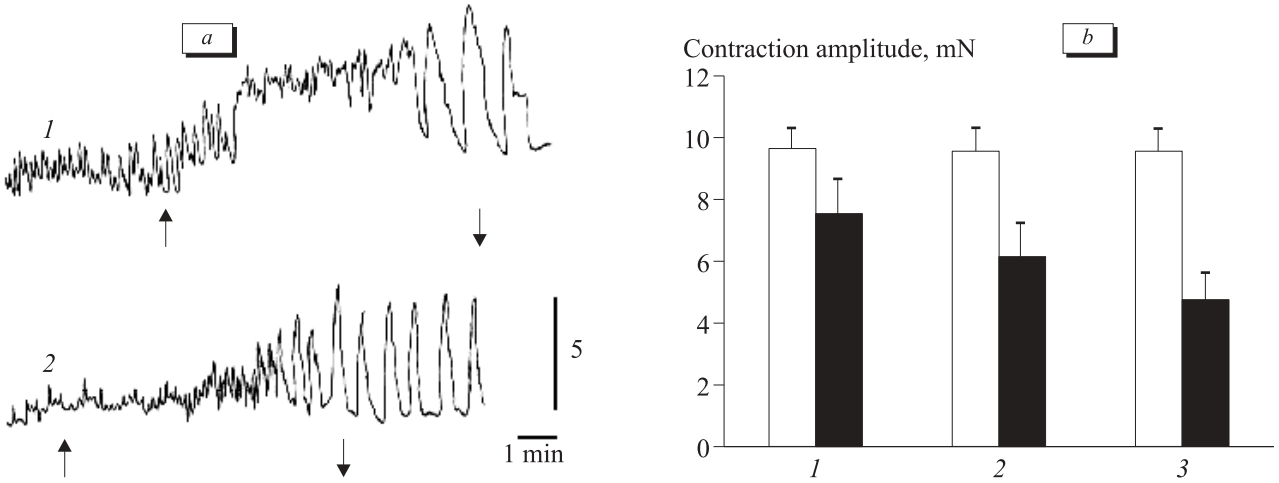

Fig. 3. Effect of Ach on isolated PV strip. *a*) contraction of PV strip preparation induced by Ach before (*1*) and during (*2*) infusion of tubocurarine. Upward and downward arrows show the start and end of Ach infusion. *b*) inhibition of Ach-induced tonic contraction of PV with various concentrations of tubocurarine: *1*) 1.5×10^{-6} M, *2*) 1.5×10^{-5} M; *3*) 1.5×10^{-4} M. Open bars: control; dark bars: experiment.

hypothesis agrees with unexpected long-term latency of the response of hepatic veins to intraportal administration of Ach (Table 1). This latency almost 3-fold surpasses the latency of BP response (Fig. 1) and the responses of these vessels to norepinephrine and sodium nitroprusside. Probably, this latent period is needed for the synthesis of vasoconstrictor factors by endothelial cells. In experiments with norepinephrine and sodium nitroprusside, which affect directly the muscle cells of the hepatic veins, the delay of PVP reaction was similar to that of BP response.

The results of special experimental series on isolated PV strips were similar: perfusion of PV with Ach solution induced a long-term constriction with the amplitude of 40% initial value (15±1 mN). De-endothelization (saponin treatment) abolished the response to Ach, but had no effect on the response to norepinephrine.

A possible involvement of endothelial nicotinic cholinergic receptors into the constrictor responses of PV was tested in experiments with tubocurarine. When injected into PV bathing solution, this toxin produced a dose-dependent inhibition of the tonic component of Ach-induced PV contractile response ($EC_{50}=1.4\times10^{-4}$ M, Fig. 3).

TABLE 1. Latencies (sec) of BP and PVP to Intraportal Injection of Ach, Norepinephrine, and Sodium Nitroprusside

Index	Ach (*n*=14)	Norepinephrine (*n*=11)	Sodium nitroprusside (*n*=10)
BP	5.0±0.6**	6.2±0.3*	5.3±0.3
PVP	14.3±2.3	3.00±0.47^{+}	5.70±0.41^{+}

Note. **$p<0.001$, *$p<0.05$ compared PVP; $^{+}p<0.001$ compared to the reaction to norepinephrine and sodium nitroprusside relatively to the response to Ach.

Nicotine, a more specific cholinergic substance affecting exclusively nicotinic receptors [2] also indicated the involvement of these receptors in Ach-induced responses of PV. When the strip preparation was perfused with nicotine solution (10^{-4} M), the amplitude of Ach-induced constriction of PV decreased from 9.04±1.08 to 6.63±0.70 mN ($p<0.01$).

For evaluation of possible involvement of neural elements of the vascular wall into Ach-induced contraction of PV, we used tetrodotoxin blocking the potential-dependent neuronal sodium channels and prevents generation and conduction of excitation waves along the nerve tissue [5]. Tetrodotoxin produced no effect on initial strength and baseline frequency of phasic contraction in PV strip preparation, but after 15 min of combined application of tetrodotoxin and Ach in the bathing solution, the strength of strip contraction decreased by 10% in comparison with the control value ($p<0.05$). This observation attests to possible involvement of PV perivenous nerves in Ach-induced reactions of PV muscular layer. Probably, Ach induced the release of norepinephrine from adrenergic nerve terminals, which contracts muscle cells in PV strip.

Thus, the vasoconstriction effect of Ach in the hepatic portal vein system is mediated via nicotine cholinoceptors located on the membrane of endothelial cells or adrenergic terminals of the hepatic veins. These cells and nerve terminals release transmitters (*e.g.* norepinephrine) affecting muscular layer of the vascular wall and induce its contraction.

REFERENCES

1. B. N. Manukhin, L. A. Nesterova, and B. K. Shaiymov, *Fiziol. Zh. SSSR*, **77**, No. 9, 102-107 (1991).
2. D. A. Kharkevich, *Pharmacology* [in Russian], Moscow (2000).
3. M. F. Shuba and N. G. Kochemasova, *Physiology of Vascular Smooth Muscle* [in Russian], Êiev (1988).
4. N. J. M. Birdsall, N. J. Buckley, M. P. Caulfield, *et al*., *The IUPHARM Compendium of Receptor Characterization and Classification,* London (1998).
5. C. J. Cohen, B. P. Bean, T. J. Colatsky, *et al*., *J. Gen. Physiol.*, **78**, 383-411 (2005).
6. S. M. Joshi, A. J. Lonigro, R. I. Secret, *et al*., *J. Pharmacol. Exp.Ther*., **259**, 171-177 (1991).
7. K. D. Macklin, A. D. J. Maus, E. F. R. Pereira, *et al*., *Ibid.,* **287**, 435-439 (1998).
8. F. Moccia, C. Frost, R. Berra-Romani, *et al*., *Am. J. Physiol. (Heart Circ. Physiol.)*, **286**, H486-H491 (2004).
9. S. Moncada, R. M. Palmer, and E. A. Higgs, *Pharmacol. Rev*., **43**, 102-142 (1991).
10. F. D. Reilly, R. V. Dimlich, E. V. Cilento, *et al*., *Hepatology*, **2**, 230-235 (1982).
11. V. A. Tsybenko, O. M. Pasichnichenko, P. I. Yanchuk, *et al*., in: *The Biology of Nitric Oxide.* Pt. 7, London (2000), p. 59.