Evidence for the Excitatory Cholinergic Innervation in the Rabbit Portal Vein

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ABSTRACT—We examined the possible existence of excitatory cholinergic innervation in isolated rabbit portal vein. Longitudinal strips of the vein in the presence of both phenoxybenzamine, an α-adrenoceptor antagonist, and N⁵-nitro-L-arginine, an inhibitor of nitric oxide synthase, exhibited a small contraction in response to transmural electrical stimulation (ES). The contractile response to ES was augmented by physostigmine, an anticholinesterase agent, and inhibited by atropine. These findings indicate the existence of an excitatory cholinergic innervation in addition to the known adrenergic and non-adrenergic, non-cholinergic innervations in the rabbit portal vein.

Keywords: Cholinergic innervation; Portal vein (rabbit), Transmural nerve stimulation

It has become evident that the blood vessel has adrenergic and cholinergic innervations as well as non-adrenergic, non-cholinergic (NANC) innervation (see ref. 1). However, it is also evident that there are remarkable regional differences in the features of innervation in the blood vessel system. Especially, the functional cholinergic neural supply to blood vessels has been a subject of controversy (1, 2). It is widely believed that the release of acetylcholine (ACh) from autonomic nerves in those tissues that receive a cholinergic innervation dilates blood vessels (see ref. 3). Cholinergic neurogenic dilatations of arteries seem to be mostly dependent on the endothelium, with some exceptions (4). On the other hand, only a few examples of mammalian blood vessels have been reported to contract in response to cholinergic neurogenic stimulation (2). Yoshioka et al. (5) have shown that canine portal vein has excitatory cholinergic innervation. However, it has not yet been demonstrated that rabbit portal vein has cholinergic innervation. The rabbit portal vein has previously been shown to have massive adrenergic and NANC innervations (6), and Brizzolara et al. (7) have very recently reported that the NANC nerve in rabbit portal vein contains both nitric oxide (NO) and ATP. Utilizing the currently available pharmacological tools, the possibility of cholinergic excitatory innervation of the longitudinal muscle of rabbit portal vein was reexamined in the present study.

Male White Japanese rabbits, weighing 2.0–3.0 kg, were anesthetized with pentobarbital sodium at the dose of 35 mg/kg, i.v. and exsanguinated. A section of the portal vein was dissected and cut into two equal longitudinal strips. In some experiments, the endothelium was removed by gently rubbing the strip with a piece of cotton wool moistened with Krebs’ bicarbonate solution. The venous preparations, about 3 mm in width and 10 mm in length, were mounted vertically between a pair of platinum electrodes separated by a distance of 5 mm, suspended in tissue baths containing Krebs’ solution aerated with 95% O₂+5% CO₂ and maintained at 37°C, and given a load of 0.5 g. The composition of Krebs’ solution was: 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2mM MgSO₄, 25.0 mM NaHCO₃ and 11.1 mM glucose. Transmural electrical stimulation (ES) was performed by means of an electronic stimulator (Nihon Kohden Kogyo, Tokyo; model SEN-3301) at an interval of 5–15 min by giving a train of 120 pulses at frequencies of 1–50 Hz. Parameters of ES were a duration of 0.3 msec and a supramaximal intensity of 25 V. The changes in tension were monitored by means of a force-displacement transducer (Nihon Kohden Kogyo, model TB-611T) and recorded on a pen-writing oscillograph (Graphtech, Tokyo; model WR-3701).

Drugs used were phenoxybenzamine hydrochloride (Tokyo Kasei Kogyo, Tokyo); guanethidine sulfate, ergotamine tartrate, acetylcholine chloride, atropine sulfate and N⁵-nitro-L-arginine (Sigma, St. Louis, MO, USA);
physostigmine sulphate (Sandoz, Basle, Switzerland); and tetrodotoxin (Sankyo, Tokyo).

Results are expressed as means±S.E.M., and “n” refers to the number of animals.

Electrical stimulation primarily produced prominent contraction of the rabbit portal vein, which was remarkably depressed by 3 × 10⁻⁸ M phenoxybenzamine, an α-adrenoceptor antagonist (Fig. 1). As shown in Fig. 1, 10⁻⁴ M N⁰-nitro-L-arginine, an inhibitor of NO synthase, potentiated the remaining small contractile response to ES, and successive 3 × 10⁻⁷ M physostigmine, an anticholinesterase agent, further augmented the contraction. Thus the augmented contractile response to ES was markedly depressed by 10⁻⁷ M atropine (Fig. 1).

When the vein was previously contracted by 1.8 × 10⁻⁸ M ergotamine, ES relaxed the vein frequency-dependently (data not shown). The relaxation was revealed to be NANC in nature (6). In contrast to the results of Brizzolara et al. (7), the NANC nerve-mediated relaxation was almost completely abolished by an inhibitor of NO synthase, but suramin, a P₂-purinoceptor antagonist, hardly affected the relaxation (Y. Mutsukado and H. Tsuru, unpublished observation). Thus, although the reason why there was a remarkable discrepancy between their experiments and ours could not be elucidated, it seems likely that ATP might not be involved in the NANC nerve-mediated relaxation under the present condition.

As shown in Fig. 2, in the presence of both 3 × 10⁻⁸ M phenoxybenzamine and 10⁻⁴ M N⁰-nitro-L-arginine, the remaining small contractile response to ES was augmented by 3 × 10⁻⁷ M physostigmine and inhibited by 10⁻⁷ M atropine. Almost all the frequency-dependent contractile responses to ES were abolished by 10⁻⁶ M tetrodotoxin. The contractile responses to ES and exogenous ACh were not influenced by rubbing the endothelium.

The ES frequency-response curve was constructed at frequencies between 1 and 50 Hz in the presence of α-adrenoceptor blockade and inhibitors of NO synthase and ACh esterase under conditions similar to those in Fig. 2. The median effective frequency (EF₅₀) was 6.5 ± 1.0 Hz (n=6) and the maximum response to ES was 72.5±2.9% of the ACh-induced maximum contraction (2.53±0.29 g, n=6).

In addition to the adrenergic innervation, Hughes and Vane (6) described NANC nerve-mediated relaxation of the rabbit portal vein, although they did not mention any transmitter candidate for the NANC nerve. In this regard, Burnstock et al. (8) proposed that ATP might be a transmitter candidate for the NANC nerve. Recently, Toda and Okamura (9) and Bult et al. (10) have shown that NO is an inhibitory NANC neurotransmitter in the cerebral artery of some mammalian species and the canine ileocolonic junction, respectively. Very recently, Brizzolara et al. (7) concluded that both NO and ATP are involved in the NANC nerve in rabbit portal vein. Indeed,
N°-nitro-L-arginine, an inhibitor for NO synthase, augmented the remaining response to ES after α-adrenoceptor blockade (Fig. 1). The augmented contractile response to ES was further potentiated by physostigmine, an inhibitor of ACh esterase, as shown in Fig. 1. This potentiation could not be shown without the inhibition of NO synthase (data not shown). The inhibition of the prominent vasodilative action of NO nerve seems to be a key to observe the excitatory cholinergic action in the portal vein of the rabbit in contrast to the dog (5).

In the presence of both an α-adrenoceptor blockade and an inhibitor for NO synthase, the remaining small contractile response to ES was augmented by physostigmine and inhibited by atropine as shown in Fig. 2. There was no difference between the intact and endothelium-denuded preparations in the response to ES. Almost all the responses to ES were completely abolished by 10⁻⁶ M tetrodotoxin. These findings indicate the existence of an

Fig. 2. Cholinergic contractile responses of isolated rabbit portal vein to electrical stimulation in the presence of α-adrenoceptor blockade and a nitric oxide synthase inhibitor. The endothelium-intact preparation was treated throughout with 3 × 10⁻⁸ M phenoxybenzamine and 10⁻⁴ M N°-nitro-L-arginine. Please note that the small contractile responses to electrical stimulation at 10, 20 and 40 Hz of 120 pulses and the response to acetylcholine 10⁻⁶ M (ACh 6) were remarkably augmented by 3 × 10⁻⁷ M physostigmine. The augmented responses to electrical stimulation (ES), but not the response to 10⁻⁷ M acetylcholine (ACh 7), were almost abolished by 10⁻⁶ M tetrodotoxin. Atropine at 10⁻⁷ M strongly depressed both the responses to 10⁻⁶ M acetylcholine (ACh 6) and ES. The upper records continue to the lower one. The agents were administered into the bath at the arrows and the bath solution was washed out at the point indicated by the symbol w. Typical recordings obtained from 7 similar experiments.
excitatory cholinergic innervation in addition to the known excitatory adrenergic and inhibitory NANC innervations in rabbit portal vein.

Brayden and Bevan (4) found that the posterior auricular artery of the cat has muscarinic receptors located directly on its smooth muscle cells that mediate smooth muscle cell relaxation, when activated by ACh released from perivascular nerves. Thus, the subtypes of muscarinic receptors located on the vascular smooth muscle cells that mediate neurogenic cholinergic dilatation and contraction remain to be characterized.

With regard to the distribution of cholinergic innervation in the circulatory system and its implication, we have presented a view that some of the differences in vascular characteristics between the two groups, i.e., one, a visceral part derived from inner tube of the embryonic body, and the other, a somatic part derived from outer tube, are of embryological origin (5). Namely, it is probable that the visceral coelomic epithelium is the common origin of the smooth muscles of both the digestive tube and the portal vein, and thus, the responses of these tissues to cholinergic impulses are in common predominantly constrictor. The present finding of excitatory cholinergic innervation in the rabbit portal vein further supports this view.

REFERENCES


