Pressor Response to Endothelin in Guinea Pigs

Liming Li, Tomohisa IShikawa, Takashi Miyauchi,
Masashi Yanagisawa, Sadao Kimura¹,
Katsutoshi Goto* and Tomoh Masaki

Department of Pharmacology and ¹Department of Biochemistry,
Institute of Basic Medical Sciences, University of Tsukuba,
Tsukuba, Ibaraki 305, Japan

Accepted February 1, 1989

Abstract—Endothelin (ET), an endothelium-derived vasoconstrictor peptide, was injected into the jugular vein (i.v.) of guinea pigs anesthetized with urethane. Blood pressure was measured from a cannula inserted into the carotid artery. All experiments were carried out after treatment with adrenergic and cholinergic antagonists. ET showed a potent, dose-dependent pressor action in guinea pigs. However, the initial, transient depressor response which is observed in rats was not produced in guinea pigs. Nicardipine (0.1 mg/kg), a dihydropyridine Ca²⁺-channel blocker, significantly inhibited the ET-induced pressor response. These results suggest that ET causes a potent pressor response, which appears to be related to the activation of Ca²⁺ channels.

Endothelin (ET) is a 21-amino-acid peptide which we recently purified and sequenced from the culture medium of porcine aortic endothelial cells (1). A human ET cDNA has been obtained from a placenta cDNA library, thereby revealing that the amino-acid sequence of human ET is identical with that of porcine ET (2). It has been reported that ET exerts a potent vasoconstrictor action on the porcine coronary artery (1) and rat renal artery (3) in vitro, and intravenously injected ET causes a potent, long-lasting pressor response in rats (1). The present study was undertaken to further evaluate the pressor action of ET through investigations of its effects on guinea pigs.

Male albino guinea pigs (300–400 g) were anesthetized with urethane (1.5 g/kg, i.p.), and the left carotid artery and the right jugular vein were catheterized with polyethylene tubing filled with 0.9% saline containing heparin (10 U/ml). Arterial blood pressure and heart rate were measured from the cannula in the carotid artery with a pressure transducer (Gould model SCK-590, Cleveland, OH) connected to a polygraph system (amplifier, Nihon Kohden AP-601G, Tokyo, Japan; tachometer, Nihon Kohden AT-601G; thermal-pen recorder, Nihon Kohden WT-687G). All experiments were carried out following the pretreatment with atropine (0.25 mg/kg, i.v.), propranolol (1 mg/kg, i.v.), and bunazosin (1 mg/kg, i.v.) in order to suppress the autonomic circulatory reflex. The intravenous injection of drugs was made through the cannula in the jugular vein. Drugs used were endothelin (Peptide Inst., Osaka, Japan); bovine serum albumin (fraction V), nicardipine (Sigma Chemical, St Louis, MO); bunazosin (Eisai, Tokyo, Japan); atropine sulfate (Tanabe, Osaka, Japan); propranolol (Sumitomo, Osaka, Japan); and urethane (Tokyo Kasei, Tokyo, Japan). ET was dissolved in a phosphate-buffered saline (pH 7.4) containing 0.05% bovine serum albumin. Appropriate vehicle controls showed no effects.

As illustrated in Fig. 1, ET produced pressor responses in a dose-dependent manner, beginning at a quite low dose of 62.5 pmol/kg. Two kinds of pressor patterns were observed: in five of 20 experiments, ET produced a monophasic, transient pressor response; and in 15 experiments, a biphasic
Fig. 1. Typical tracing of the effects of ET on pulsatile arterial pressure and heart rate in guinea pigs. ET was introduced by i.v. bolus injection, noted by the dots. Each number represents an agonist dose (pmol/kg). BP: blood pressure. The dose-dependent pressor responses to ET were divided into two groups: one was monophasic and transient at all doses (upper panel) and the other was biphasic consisting of a transient response and a subsequent slowly-developing and long-lasting one at high doses (lower panel).

response was observed that consisted of a transient response followed by a slowly-developing and long-lasting one. On the guinea pig femoral artery, ET induces a contraction in some but not all the preparations (4). Such individual differences in vasocontractile response to ET may contribute to the two kinds of pressor patterns. Small changes of heart rate were also induced by i.v. injected ET (data not shown). However, no regular patterns were recognized in them. In some experiments, ET exerted a positive chronotropic effect, which is consistent with the in vitro study using the isolated guinea pig right atria (5). However, there was no dose-dependency, and the amplitude of increase in heart rate was smaller than that in the in vitro study. In fact, there was even a decrease in heart rate by i.v. injection of ET in some experiments. The studies using Langendorff's method have demonstrated that ET induces a potent coronary vasoconstriction on the perfused heart of rats (6) and guinea pigs (T. Ishikawa, unpublished data). The ischemia of the heart which results from the potent coronary vasoconstricting effects of ET may be the reason for the disappearance of a noticeable increase in heart rate. The injection of a higher dose, 2000 pmol/kg, lead to the fatality of all the examined animals.

Figure 2A shows the dose-response relationship for the pressor response to ET. The ED50 of the pressor response to ET was estimated to be in the range of around 200 pmol/kg, suggesting that ET is one of the most potent pressor substances. The pressor response to ET was markedly inhibited by nicardipine, a dihydropyridine Ca\(^{2+}\)-channel blocker (Fig. 2B). Considering that the vasoconstrictor effects (1) and the cardio-tonic effects (7) of ET are also sensitive to nicardipine in vitro, it is suggested that the pressor effect of ET in vivo results from a direct action on the vascular smooth muscle as well as the cardiac muscle through a mechanism closely related to the activation of Ca\(^{2+}\)-channels.

It has been reported that ET produces changes in the blood pressure of anesthetized rats, which is composed of three phases: an initial depressor, a rapid and transient pressor, and a subsequent long-lasting pressor response (1). Different from the effects in rats, ET produced no depressor response in guinea pigs. It has not yet been indicated whether the depressor response is due to a direct action of ET or an indirect action, i.e., ET facilitates the release of vasodilator substances such as EDRF. Furthermore, with a dose of 1000 pmol/kg, the pressor response to ET lasts more than 2 hours in rats (1), whereas in guinea pigs, there was a recovery
Fig. 2. Pressor action of ET and influence of calcium channel blocker. A. Dose-response relationships for ET-induced rises in amplitude of mean arterial pressure. Each point represents the mean±S.E.M. of 9 experiments. B. Influence of nicardipine on the pressor response to ET (500 pmol/kg). Nicardipine (0.1 mg/kg) was intravenously injected 10 min before the administration of ET. Each column represents the mean±S.E.M. of 8 experiments. *P<0.01 for the significant difference (Student’s t-test for paired values).

to the base line within 30 min. These results imply that differences do exist between rats and guinea pigs in regard to the effects of ET in vivo. Recently, the rat ET gene has been cloned and sequenced. Rat ET is a 21-residue peptide, which differs a little in the amino acid sequence from porcine/human ET (6). Rat ET also has a vasoconstrictor activity. However, the activity of rat ET on rat aortic strips is less potent than that of porcine/human ET. Moreover, on rat perfused hearts, rat ET-induced coronary vasoconstriction returns to the base line level within 30 min in contrast to porcine/human ET which induces a long-lasting response. Such differences in the effects of rat ET and porcine/human ET could be explained by not only the differences in the amino acid sequence but also an existence of each specific receptor. Therefore, if a guinea pig-type ET and its specific receptor exist, it would be a clue for elucidating the possible reasons for the differences of the responses to porcine/human ET between rats and guinea pigs. The ET receptor has been characterized in cultured rat aortic vascular smooth muscle cells (8), but it is still a matter of controversy.

Prepro-ET mRNA is expressed not only in the cultured endothelial cells but also in aortic endothelium in vivo (1), suggesting that ET is an endogenous substance and may play an important role in the cardiovascular regulation along with angiotensin II and norepinephrine. It is of particular interest that the pressor response induced by ET lasts much longer than that by angiotensin II or norepinephrine. Since the pressor response to angiotensin II or norepinephrine was transient and very short (data not shown), these substances need to be released all the time in order to maintain the blood pressure at a high level. On the contrary, once ET is released, the blood pressure will be continuously elevated. The obvious morphological changes of endothelium have been reported in hypertensive animals (9). Although the mode of ET release has not been made clear yet, it can be speculated that a subtle change of endothelium, either functionally or morphologically by some means, may lead to the release of ET since ET is released in large quantities under the culture conditions that can produce abnormal stimulations on endothelial cells (1). Furthermore, ET-induced pressor response is sensitive to dihydropyridine Ca\(^{2+}\)-channel blockers which are widely used as antihypertensive agents. Taken together, it can be suggested that the
potent and sustained pressor action of ET may be closely related to a certain type of hypertension.

Acknowledgments: The authors thank Ms. Lisa G. Bond for advice in preparation of the manuscript. This work was supported by grants from the University of Tsukuba Project Research and Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References


