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Vasoactivity of endothelin in nasal blood vessels

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SUMMARY

Endothelin (ET) is a newly described peptide that was isolated from the supernatant of cultured porcine aortic endothelial cells. Among the three isoforms of ET, the originally discovered ET-1 is the only one that exists in vascular endothelium. It is reported to be an extremely potent vasoconstrictor in a variety of blood vessels. Using in vitro bioassay technique, the vasoactivity of ET-1 in both canine and human nasal mucosa was investigated.

ET produces a contraction which is slow in onset and sustained in the blood vessels both of the canine and the human nasal mucosa. The threshold of ET-1 in causing contraction was $10^{-9}M$ (dog) and $10^{-8}M$ (human).

This response was turned out to be extracellular Ca^{2+}-dependent, because either Ca^{2+}-free medium or application of nifedipine almost abolished it. A subthreshold concentration of ET-1 enhances exogenously applied noradrenaline (NA)-induced contractions, but not endogenous NA-induced contractions caused by transmural electrical stimulation. As endothelium derived substances, such as ET-1 showed strong vasoactivity, endothelium may play an important role in maintaining vascular tension of the nasal blood vessels along with neural control.

INTRODUCTION

Vascular tone means an active sustained contraction of vascular smooth muscle not demonstrably dependent on stimuli from outside the muscle as described by Mellander and Johnson (1968). Although the mechanism for the development of inherent tone has not yet been clarified, it can be conceived of as the integrated result of phasic twitches initiated by action potentials of smooth muscle cells. Continuous release of noradrenaline (NA) from the sympathetic nerve terminal with the inhibition of its release by acetylcholine (ACh), which is secreted from

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parasympathetic neurons, has been regarded as a major part of maintaining vascular tone (Jackson, 1982). The vigorous work with regard to coexistence of neuropeptides in neurons along with classic neurotransmitters has elucidated their role as co-transmitters or modulators in neuromuscular junctions. The candidates for these modulators are neuropeptide Y (NPY) in the sympathetic neuron and vasoactive intestinal polypeptide (VIP) in the parasympathetic nerve. The experiments by Furchgott and Zawadski (1980) showed that the presence of endothelial cells is essential for ACh to produce relaxation of vascular smooth muscle. Abundant data have accumulated suggesting that endothelium may function to release vasoactive substances (EDRF: endothelium-derived relaxing factor and PGI_2: prostacyclin) and modulate the hemodynamic actions of the muscles. A vasoconstrictive agent released from endothelial cells in response to anoxia, stretch, increased transmural pressure and several other stimuli has also been reported by De Mey and Vanhoutte (1983) under the term of endothelium-derived contracting factor (EDCF). At least two types of EDCFs, peptide and prostanoid, have been postulated by the subsequent works.

Thus, in addition to the classic transmitters NA and ACh, works in the last decade has introduced NPY, VIP, EDRF, PGI_2, and EDCFs to the list of transmitters and modulators that control nasal blood vessels. Now, we will describe our work with endothelin.

Endothelin (ET) is a newly described peptide that was isolated from the supernatant of cultured porcine aortic endothelial cells and was determined its amino-acid sequence by Yanagisawa et al. (1988). Since then, ET has gained remarkably increased recognition.

ET is a 21 aminoacid peptide with three isoforms for which three separate genes have been cloned. They are called the human ET family (ET-1, 2, 3) (Inoue et al., 1989). ET-1 is an original ET and was formerly termed porcine/human ET. Its binding sites have been found not only on vascular smooth muscle but also on other organs including brain, adrenal gland, kidney, lung, intestine, uterus, pancreas, thyroid gland, etc.

The only ET that exists in vascular endothelium is ET-1, whose reactivity is from 10 to 100 times stronger than ET-3, but is weaker than ET-2 (Inoue et al., 1989). ET-1 has been shown to be released from vascular endothelial cells in vitro. Substances that promote phosphate-inositol turnover, such as adrenaline and thrombin, have been reported to increase the expression of the messenger RNA for endothelin's precursor, preproendothelin by Yanagisawa et al. (1988). However, little is known as to the physiological stimuli for the release of ET-1. ET-1 has been reported to contract the smooth muscle in a variety of isolated blood vessels (Yanagisawa et al., 1988). The potency of ET-1 as a vasoconstrictor has been shown to be greater than that of other known constrictor agents, including NA.

The mechanism of maintaining vascular tone seems to be too complicated to elucidate at this point.

However, what we can do at present is to examine the response of the blood vessels to various vasoactive substances in a variety of conditions. We have already reported in vitro vasoreactivities of NA and several α-agonists (Ichimura and Jackson, 1984; Ichimura and Chow, 1988), ACh (Seki et al., 1986), and several neuropeptides (Ichimura et al., 1988) in nasal blood vessels. The aim of this study is to show the in vitro vasoactivity of ET-1 in both canine and human nasal mucosa and to investigate the possibility of the dynamic role of endothelium as a modulator of the vascular tone in nasal mucosa.

METHOD

Human specimens were obtained during turbinectomies performed on patients complaining of severe nasal obstruction due to nasal allergy or hypertrophic rhinitis, while canine specimens were taken from nasal septum of sacrificed mongrel dogs. Nasal mucosa, stored in aerated Krebs solution, was cut into pieces approximately 5 × 15 mm. A piece was suspended in a muscle bath containing 10 ml of Krebs solution. The strip of mucosa was fixed at the lower end of the bath and the upper end was attached to a strain gauge transducer with 3-0 silk sutures under a tension of 0.5 g. The bath solution was constantly gassed with 95% O_2 and 5% CO_2. The tension was allowed to equilibrate for about 40 minutes, then its changes were recorded isometrically.

The tissue contracts when treated with vasoconstricting agents. Firstly, the tissue was stimulated with 10^{-5}M or 10^{-4}M NA to examine if its contractility was strong enough. Then, the following experiments were performed:

1. The response to increasing concentrations of ET-1

Concentration-response curves for the contractile effects of ET-1 were obtained by cumulative addition of the peptide to the organ bath at 30 min intervals.

2. The effect of antagonists of known receptors which mediate vasoconstriction

The same procedure was done as 1) with pretreatment of phenoxybenzamine (POB), an α-adrenergic antagonist with α_1 selectivity, yohimbine (YOH), an α-adrenoceptor antagonist with α_2 selectivity, pyrilamine (PYR), an H_1-histaminergic antagonist, and atropine (ATR), a muscarinic antagonist. Histamine at doses higher than 10^{-4}M caused canine tissues to contract, but relaxed human nasal mucosa. Therefore, the PYR inhibition study was performed only in canine tissues.

3. The effect of ET-1 on tissues under sustained contraction

Methoxamine (MX) caused the mucosa to contract protractedly. ET was introduced after the contraction reached a plateau.

4. The contribution of the influx of extracellular calcium ion (Ca^{2+}) to the ET-induced contraction

Experiments were conducted in a Ca^{2+} free medium with EGTA or in the presence of the Ca^{2+} blocker nifedipine (NIF).

5. The effect of ET-1 upon electrically induced contraction

Electrical stimulation produced with a SEN 3301 stimulator (Nihon Kohden) was applied transmurally by monophasic pulses at 5 Hz, 5 sec, 40 V at 1.5 min intervals. This stimulation induces NA release from the adrenergic nerve terminal. The released NA causes a muscle contraction. After the magnitude of the response became stable, increasing concentrations of ET-1 were introduced into a bath to observe the magnitude of the subsequent electrically-induced responses.

6. The effect of ET-1 on exogenously applied NA-induced contraction

10^{-5}M NA produced a contractile response. After the contractile responses became reproducible, a subthreshold concentration of ET-1 was introduced before the introduction of NA in order to see if ET-1 affects the subsequent NA-induced responses.

Drugs used in this study were as follows: noradrenaline bitartrate (NA, Sigma), endothelin-1 (ET, Peptide Institute), phenoxybenzamine hydrochloride (POB, Tokyo-Kasei), yohimbine hydrochloride (YOH, Sigma), pyrilamine maleate (PYR, Sigma), atropine sulfate (ATR, Sigma), EGTA (Sigma), nifedipine (NIF, Bayer), methoxamine hydrochloride (MX, Nihon-Shinyaku), dl-propranol hydrochloride (Sigma), ascorbic acid (Sigma).

RESULTS

1. The response to increasing concentrations of ET-1

ET-1 caused concentration-dependent contractions from 10^{-9}M in the canine specimens (N=5), and from 10^{-8}M in the human tissue (N=4) (Figure 1). Half maximal contractile responses (EC_{50}) were evoked by 10^{-7}M ET-1 (dog) and 3×10^{-7}M ET-1 (human) respectively, a concentration somewhat higher than that reported earlier in other vessel specimens (Ichimura and Jackson, 1984; Ichimura and Chow, 1988; Ichimura et al., 1988).

The speed of the contraction induced by ET-1 was considerably slower than that by other vasoconstrictors, such as NA. For example, in human nasal mucosa it took more than 120 minutes to reach maximal contraction with 10^{-7}M ET-1, while it took 16 minutes with 10^{-4}M NA. ET-induced contraction was maintained for a long time, and its plateau curve continued more than six hours in two of five canine tissues. It did not return to baseline even 12 hours after washing out ET-1 from the organ bath. Subsequent responses to ET-1 were absent in the specimen.

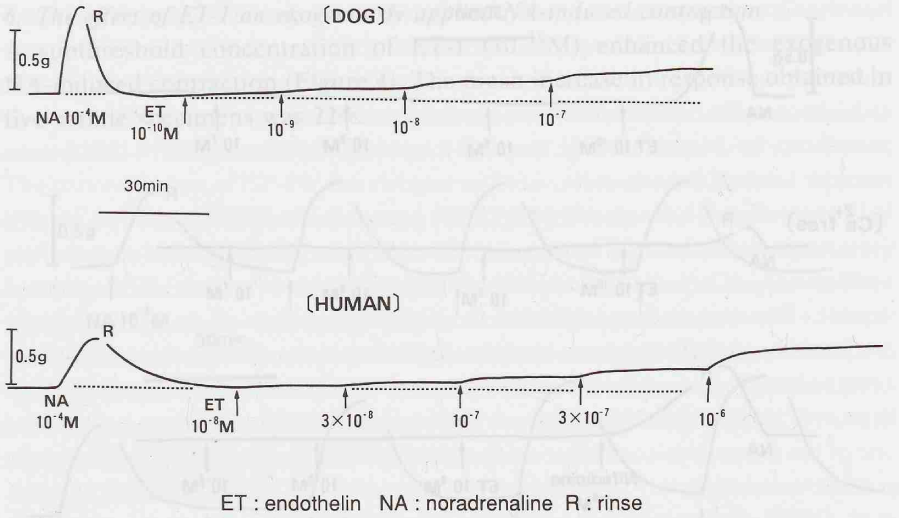

Figure 1. Contractile responses induced by endothelin-1.
upper: canine septum mucosa, lower: human turbinate mucosa.

The responses obtained in human specimens were slower and smaller compared with that of canine tissues.

2. The effect of antagonists of known receptors which mediate vasoconstriction
While 3×10^{-8}M POB was able to attenuate the NA-induced contraction as described before by the authors (1988), it did not inhibit the ET-induced contraction. ET-induced contraction was also resistant to YOH, PYR, or ATR. This suggests that the contraction is not mediated by the well-known receptors which may induce vasoconstriction, such as α-adrenergic, H_1-histaminergic, and muscarinic receptors, but it occurred by stimulation of the smooth muscle cell, possibly through its own receptor.

3. The effect of ET-1 on tissues under sustained contraction
ET-1 at 10^{-9}M caused a further contraction when the nasal mucosa was submaximally contracted by 10^{-5}M MX.

4. The contribution of the influx of extracellular Ca^{2+} to the ET-induced contractions
ET-induced contraction in human tissue was abolished in the lack of Ca^{2+} in the medium, and pretreatment with 10^{-6}M the calcium influx blocking agent NIF completely inhibited the cumulative concentration-response curve for ET-1 (N=3: dog) (Figure 2). Addition of Ca^{2+} restored the contraction. There was no difference in Ca^{2+}-dependence between species.

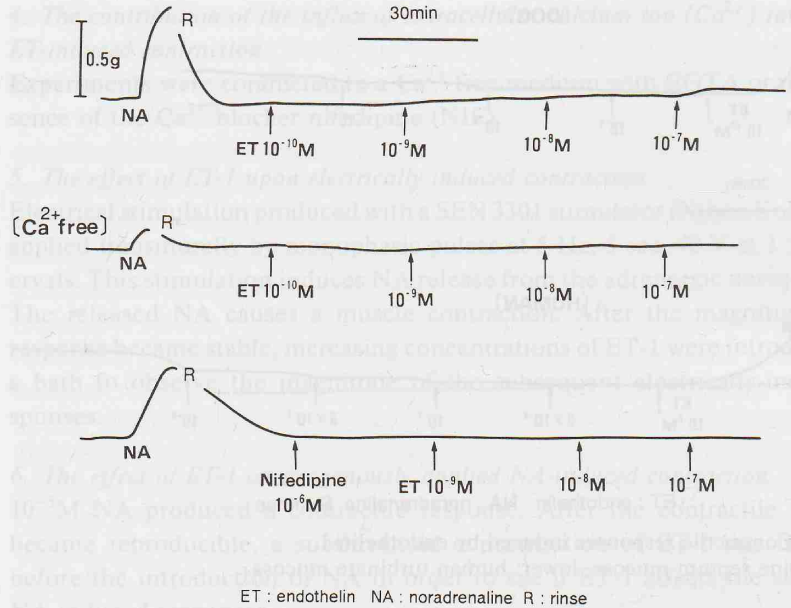

Figure 2. Endothelin-induced contraction with and without calcium ions (Ca^{2+}) in the bath solution, and with nifedipine treatment.
upper; Ca^{2+} 2.5mM, middle; Ca^{2+} OmM + EGTA, lower; Ca^{2+} 2.5mM + nifedipine.

5. The effect of ET-1 upon electrically induced contraction

ET-1 did not affect endogenous NA-induced contraction produced by transmural electric stimulation until the concentration reached 10^{-7}M, where the baseline was elevated by the characteristic vasoconstricting effect of ET-1 (N=5: dog, N=2: human) (Figure 3).

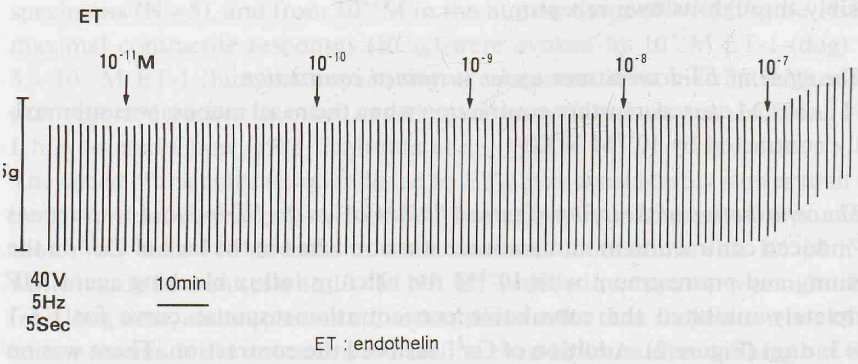

Figure 3. Effect of endothelin-1 upon electrically-induced contraction.

6. The effect of ET-1 on exogenously applied NA-induced contraction

A subthreshold concentration of ET-1 (10^{-10}M) enhanced the exogenous NA-induced contraction (Figure 4). The mean increase in response obtained in five canine specimens was 22%.

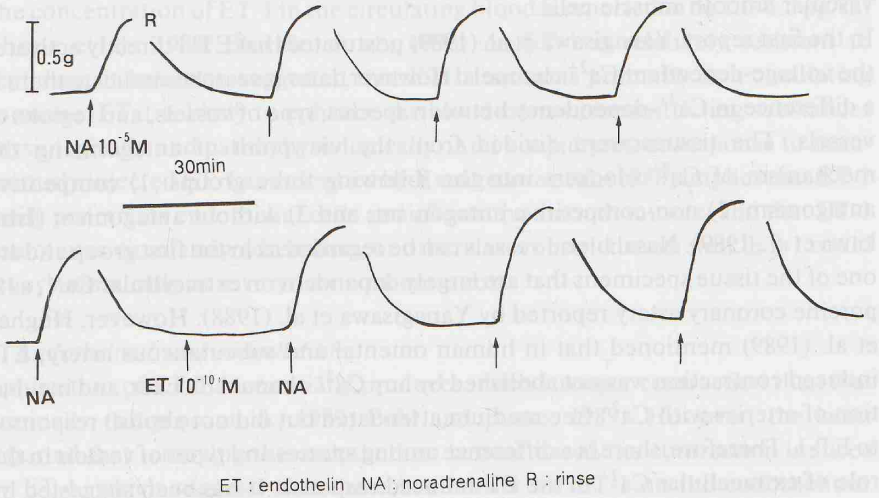

Figure 4. Effect of endothelin-1 upon contraction induced by exogenously-applied noradrenaline (NA).

DISCUSSION

1. Endothelin-1 as a vasoconstrictor

It has been demonstrated that ET-1, the most potent vasoconstrictor known to date reported by Yanagisawa et al. (1988), is also an extremely potent vasoconstrictor in nasal blood vessels with an EC_{50} of less than 10^{-7}M. This potency is about 1000 times more than NA. This is compatible with the report by Fortes et al. (1989) that ET-1 was 1000 times more potent than NA in constricting both arterioles and venules in the exteriorized rat mesentery in situ. The ET-induced contractions that occurred in nasal blood vessels were slower in onset than those seen with the other modes of activation, but reached a statistically equivalent maximum tonic level (at 3×10^{-6}M) as compared with NA (3×10^{-4}M). ET-induced contractions of the nasal vasculature were long-lasting compared with any other vasoconstrictor examined so far except for prostaglandins (Jackson and Birnbaum, 1982), and were characteristically difficult to wash out. A long-lasting desensitization specific to ET-1 is suggested by the observation that no subsequent response to ET-1 could be obtained even at several hours after the wash-out. However, responses to other contractile agonists remained intact after ET-1 wash-out.

It is widely accepted that an increase in free intracellular Ca^{2+} is essential for the muscle contraction. Removal of extracellular Ca^{2+} reduced the ET-induced contraction. Moreover, NIF completely suppressed the response, indicating that its blocking effect is complete when nasal mucosa is stimulated by ET-1. It was postulated by Masaki (1989) that ET-1 acts by facilitating Ca^{2+} influx into vascular smooth muscle cells.

In the first report, Yanagisawa et al. (1989) postulated that ET-1 directly activates the voltage-dependent Ca^{2+} channels. However, later researches indicate there is a difference in Ca^{2+}-dependency between species, type of vessels, and regions of vessels. The tissues were divided from the viewpoint of antagonizing the mechanism of Ca^{2+} blockers into the following three groups: 1) competitive antagonism; 2) non-competitive antagonism; and 3) without antagonism (Ishikawa et al., 1989). Nasal blood vessels can be regarded as in the first group and are one of the tissue specimens that are largely dependent on extracellular Ca^{2+}, as is porcine coronary artery reported by Yanagisawa et al. (1988). However, Hughes et al. (1989) mentioned that in human omental and subcutaneous artery, ET-induced contraction was not abolished by any Ca^{2+} channel blocker, and incubation of arteries with Ca^{2+} free medium attenuated but did not abolish responses to ET-1. Therefore, there is a difference among species and types of vessels in the role of extracellular Ca^{2+} in the ET-induced response. It has been suggested by Ishikawa et al. (1989) that ET-1 stimulates the L-type Ca^{2+} channel and induces the inward Ca^{2+} current and contraction.

Because of the desensitization described above, namely, slow return from the first contraction and inactive response to the second challenge, the inhibition study could not be performed after the first challenge of ET-1. Hence, the experiments were conducted paired specimens taken from the adjoining part of the nasal mucosa, one specimen for the control and the other for the NIF study or the Ca^{2+}-free medium study. Experiments could be carried out if both specimens showed almost the same response to the application of 10^{-5}M NA.

2. Endothilin-1 as a potentiator

According to the experiment performed by Wiklund et al. (1988) using guinea-pig femoral artery, ET-1 (10^{-10}–10^{-8} M) inhibited the fractional release of [^{3}H] NA induced by transmural nerve stimulation in a dose-dependent fashion. This suggested that ET-1 can act as a potent neuromodulator of adrenergic neuroeffector transmission. What we measured in this study was not NA release, but it was the resultant NA-induced contractile response. Our data showed that ET-1 did not affect the electrically-induced response and was not in accordance with their conclusion. The discrepancy might be due to the difference between species or type of vessels.

Our result showing that a subthreshold concentration of ET-1 enhances

exogenously applied NA-induced contractions but not endogenous NA-induced contraction may be a matter of concern if we stand a viewpoint that ET-1 has a physiological role in maintaining vasomotor control. Namely, ET-1 is proved to have postjunctionally mediated mechanisms in the nasal blood vessels, which is agreed well with the result by Han et al. (1990) using mesenteric artery of the rat. The concentration of ET-1 in the circulating blood is reported to be 1.0–1.5 pg/ml (Hirata et al., 1989; Shichiri et al., 1990) As ET-1 is relativety resistant to peptidase, it has been suggested that ET-1 functions primarily as a circulating hormone. ET-1 is likely to enhance the blood-born vasoconstricting substances physiologically and pathophysiologically. And a rapid pressor response to intravenously injected ET-1 as described by Yanagisawa et al. (1988) implies that ET-1 easily penetrates endothelial cells and affects the smooth muscle, and that ET-1 potentiates circulating adrenaline, or other vasoconstrictors as well. ET-1 could act systematically at a very low concentration.

3. Endothelin-1 and endothelium

Application of ET-1 adventitially produces much stronger contraction than that luminally (Shigeno et al., 1989; Pohl and Busse, 1989). However, the latter reported that removing the endothelium has increased the contraction induced by ET-1, which suggests that there is a barrier for ET-1 on the endothelium. Moreover, an autoradiographic study by Dashwood et al. (1989) demonstrated that the receptors for ET-1 in the muscle layer lay near the adventitia. So, in the in vitro study, ET-1 might produce its effect adventitially.

In our former in vitro bioassay technique with nasal mucosa specimens (Seki et al., 1986) endothelium-derived relaxation was not consistently demonstrated in nasal mucosa even when using ACh. This might be due to the difficulty in preserving the endothelium when we prepare the specimens. It has been shown that an intact endothelium is a prerequisite for vasodilation induced by a number of stimuli, including ACh.

Injury of the endothelium could result in loss of the barrier for ET-1 as well as hormones and autacoids in the blood. The result is an increase in vasoactivity for these substances, a decrease in producing EDRF and PGI_2, and a defect in regulating neural vasomotor control.

It is postulated that as endothelium-derived substances, such as ET-1, showed a powerful vasoreactivity, endothelial cells may play an important part in the regulation of vasomotor tone of nasal blood vessels, along with the autonomic neural control mechanism. Further studies are required to elucidate the pathophysiological significance of the region-specific effects of ET-1.

CONCLUSION

1. ET-1 produces an extracellular Ca^{2+}-dependent contractile response, slow in onset and sustained, in the blood vessels both of the canine and the human nasal mucosa.
2. The threshold of ET-1 in causing contraction is the lowest of the drugs examined so far.
3. A subthreshold concentration of ET-1 enhances exogenously applied NA-induced contractions, but not endogenous NA-induced contractions. This might be related to the hormonal effect of ET-1.
4. As ET-1 has an endothelial origin and showed strong vasoactivity, endothelium may play an important role in maintaining vascular tension along with neural control.

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