Facial Vein of the Rabbit

Neurogenic Vasodilation Mediated by β-Adrenergic Receptors

BARBARA L. PEGRAM, PH.D., ROSEMARY D. BEVAN, M.D., AND JOHN A. BEVAN, M.D.

SUMMARY A segment of the facial vein of the rabbit, that opposite the buccal cavity, responds to norepinephrine (NE) and transmural nerve stimulation (TNS) by a brisk biphasic dilation. The dilation in response to both procedures is reversed by prior exposure to propranolol (10⁻⁶ M). Pretreatment with phenoxybenzamine (10⁻⁵ M) increases the size of the neurogenic response and displaces the NE dose-relaxation curve to the left. Histamine causes a constrictor response exclusively. Sympathetic stimulation of a segment of the facial vein proximal to this buccal segment, and also of the external jugular vein, results in constriction. Light microscopy showed no features which can account for the dilation, and fluorescence histochemistry using a modified Falck technique showed a dense adrenergic nerve plexus extending throughout the thickness of the media. We found that frequency-response characteristics and neuronal uptake of ⁶⁷⁷H-NE were consistent with findings for a blood vessel with a heavy medial innervation. Also, monoamine oxidase and catechol O-methyltransferase activities were similar to those found in other rabbit veins. Furthermore, these results are consistent with an adrenergic neurotransmitter organization in which there is a predominance of β over α-adrenergic receptors. In conclusion, the presence of a dilator response in this buccal segment of the facial vein may be related to its location in the wall of the cheek, where it may be subjected to considerable stretch.

THE PRESENCE of both α- and β-adrenergic receptors in vascular smooth muscle is undisputed. In most vascular preparations, however, the β effect of norepinephrine (NE) and neuronal activity is seen only after α-adrenergic receptor blockade. In other vessels the vasoconstriction induced by NE is increased after β-receptor blockade. Autoregulatory escape can also be inhibited partially by β-adrenergic receptor blocking agents. Although venous dilation can be brought about through β-adrenergic receptor activation, most isolated venous preparations contract in response to NE (e.g., see Bevan et al.). During the course of a survey of the adrenergic parameters of a number of rabbit veins, it was noted that, in contrast to all other veins studied, part of the facial vein responded to adrenergic stimuli with a biphasic relaxation. On a morphological study no features were found that would distinguish this part of the vein from portions on either side which contracted in response to stimulation. This study is concerned with the adrenergic properties of the relaxing part of this vein—the buccal segment of the anterior facial vein of the rabbit.

Methods

After exsanguination of albino rabbits, segments of the external jugular and the facial veins were removed and placed in Krebs’ bicarbonate solution gassed with 95% O₂ and 5% CO₂. The segments of the facial vein used in this study lie subcutaneously on the outside of the wall of the cheek. The vein is dissected from the lower border of the mandible to the confluence of the small veins that drain the orbit and its surroundings, and is placed in a tissue bath; the appropriate segment (that anteromedial to the maseter muscle) is removed for examination (Fig. 1). Extraneous tissue was removed by dissection with the aid of a Zeiss dissecting microscope.

HISTOLOGY AND FLUORESCENCE HISTOCHEMISTRY

Facial veins from a number of rabbits were removed from their origin at the confluence of smaller veins at the lower border of the mandible and fixed in formaldehyde. Transverse sections (5 μm) were cut and stained with hematoxylin and eosin, Verhoeff’s elastin stain, and Gomori trichrome and examined by light microscopy. Other specimens were processed according to the glyoxylic acid fluorescence method of Lindvall and Borklund. The veins were immersed in a 2% glyoxylic acid solution in which the pH had been adjusted to 7.5–8.5 for 3–5 minutes at 0°C. They were then blotted, frozen rapidly in isopentane chilled with liquid nitrogen, and freeze-dried for 24 hours. After freeze-drying, the venous segments were heated in an oven at 100°C for 6 minutes to intensify the fluorescence. The specimens were vacuum-embedded in paraffin and serial sections were cut at 10 μm along their length. Tissues were observed using a large Zeiss fluorescence microscope illuminated by a 250 mercury vapor lamp, a 3-mm BG3 excitation filter, and a 500-mm barrier filter. Photographs were taken with a Zeiss-Ikon camera using Kodak Tri-X film.

CONTRACTILE RESPONSE TO EXOGENOUS NOREPINEPHRINE AND TRANSMURAL NERVE STIMULATION

Ring preparations of veins 5 mm in length were set up in vitro as previously described. Rings were connected to a Statham G10b (±0.15 ounces) strain gauge, and their contractile responses to exogenous NE, isoproterenol, and histamine and transmural nerve stimulation (TNS) were monitored on a Grass model 5 polygraph. A resting tension of 500 mg, previously shown to be optimum, was applied to each vessel. After an equilibration period of 30
minutes, a steady state contractile response to a median effective dose (ED₅₀) of the agonist was obtained. The tissue then was washed and allowed to recover for 10 minutes. This procedure was repeated until the response to the agonist was consistent. Noncumulative, bracketed responses to various doses of agonists were elicited to provide data for dose-response curves. By similar means, dose-response curves for NE and isoproterenol were determined in the presence of propranolol (10⁻⁶ M), and for NE in the presence of phenoxybenzamine (10⁻⁶ M).

Two platinum electrodes placed parallel to the long axis of the vein ring were used for TNS. Pulses of 0.3-msec duration and supramaximal voltage were found satisfactory to selectively release endogenous transmitter without stimulating the smooth muscle directly. Steady state contractile responses to TNS applied at different frequencies were bracketed with responses at 8 Hz to obtain the frequency-response relationship. TNS also was carried out in the presence of propranolol (10⁻⁶ M) or phenoxybenzamine (10⁻⁶ M).

**UPTAKE OF ³H-NE AND ¹⁴C-SUCROSE**

The uptake of tritiated l-norepinephrine (³H-NE) and ¹⁴C-sucrose were measured as previously described.³ Pairs of longitudinal strips of the buccal segment of the facial vein were equilibrated for 1 hour. One of each pair then was exposed to cocaine (10⁻⁴ M) for an additional 30 minutes. All strips were incubated in ³H-NE (3.3 μCi:10⁻⁸ M) and ¹⁴C-sucrose (20 μCi:2 × 10⁻⁴ M) for 1 hour. The strips were removed from the bath, rinsed for 1 second in Krebs' bicarbonate solution, blotted, weighed on a Cahn electrobalance, and then digested overnight in Soluene 100 (Packard). The quantity of each isotope present in the strips was determined by standard liquid scintillation procedures.

The uptake of ³H-NE and ¹⁴C-sucrose was expressed as milliliters of bath fluid cleared per gram of tissue. The uptake of ¹⁴C-sucrose represents the uptake into the extracellular space. It was assumed that an equivalent amount of ³H-NE also was present in the extracellular space. Extraneuronal uptake of ³H-NE was taken to be the uptake in the presence of the neuronal uptake blocking agent, cocaine, minus that into the sucrose space. The cocaine-sensitive component of uptake was equated with neuronal uptake.

**ENZYME ASSAY**

The method described by Wurtman and Axelrod⁴ was used to determine monoamine oxidase (MAO) activity in the anterior facial vein. Catechol O-methyltransferase (COMT) activity was measured by the method of Krakoff et al.¹⁰ In the presence of MAO, ¹⁴C-tryptamine is converted to ¹⁴C-indolyacetic acid. This compound is then extracted into toluene-isoamyl alcohol mixture (3:2). ¹⁴C-labeled S-adenosylmethionine and l-norepinephrine form ¹⁴C-normetanephrine in the presence of COMT. This compound is extracted into toluene-isoamyl alcohol mixture (3:2). Standard liquid scintillation procedures were used to determine the quantity of ¹⁴C-indolyacetic acid and ¹⁴C-normetanephrine formed.

**STATISTICS**

Student's t-test was used for statistical analysis of the data; differences were considered significant when P < 0.05.

**Results**

**HISTOLOGY AND FLUORESCENCE HISTOCHEMISTRY**

Transverse sections of the portion of the facial vein used in this study showed that it consists of three layers, endothelium and media which merge into adventitia with no distinct boundary zone (Fig. 2A). No elastin could be demonstrated in the wall by Verhoeff's elastin stain. Endothelial cells have well defined basement membranes and are frequently separated from medial smooth muscle cells by small collagen bundles. The media consists of smooth muscle cells, mostly circularly orientated and separated into layers and bundles by collagen, which increases in amount toward the adventitia. In the adventitia small groups of smooth muscle cells are seen among the collagen bundles, frequently orientated obliquely or longitudinally. The adventitia contains fibroblasts and is relatively vascular.

Fluorescence microscopy demonstrated a dense adrenergic innervation, evenly distributed throughout the vessel wall, and single varicose fibers were frequently seen to extend to the endothelium (Fig. 2B).

**TRANSMURAL NERVE STIMULATION**

Electrical transmural stimulation of the intramural nerves (TNS) of the buccal segment of the facial vein at a frequency of 8 Hz resulted in a biphasic response (Fig. 3). This was similar in pattern to the contractile response previously reported for the rabbit ear artery.¹¹ It consisted of an initial rapid relaxation, which occurred after a latency of only 1-2 sec (phase A), followed by a transient contraction and a second more slowly attained tonic phase (phase B) of relaxation. The entire response was completely prevented by prior treatment of the preparation with tetrodotoxin (0.6 μM). This pattern of response was seen consistently throughout the duration of an experiment. Upon cessation of TNS the tissue rapidly regained its original tone although sometimes a transient increase in tone above this level was observed. However, in the pres-
Figure 2  A: photomicrograph of transverse section through wall of rabbit facial vein stained with hematoxylin and eosin. E = endothelium; M = media; A = adventitia; C = collagen; S = smooth muscle cell. (For description see text.) B: fluorescence micrograph of transverse section of buccal segment of rabbit facial vein. Note that varicose adrenergic fibers are evenly distributed throughout the wall and extend to the endothelium.
ence of the β-adrenergic blocking agent propranolol (10⁻⁶ M), which by itself did not alter the resting tone of the tissue, the previously observed relaxation upon TNS was reversed and a contractile response was seen (Fig. 3).

The control peak response to TNS at 16 Hz, expressed as a percentage of the maximum relaxation induced by exogenous NE, was 46% for both phases (Table 1). In the presence of the α-adrenergic blocking agent phenoxybenzamine (10⁻⁶ M), phase B of the neurogenic response was increased to a mean of 91.8% of the maximum relaxation to NE. The contractile response to TNS observed in the presence of propranolol was 47.1% of the maximum contraction to NE (Table 1).

Confirmatory evidence that the neurogenic response of the facial vein is mediated via adrenergic nerves was provided by studies with 6-hydroxydopamine. After exposure to this drug (2-5 x 10⁻⁶ M) for 2 hours, the response of the facial vein to TNS essentially disappeared. Under the same conditions the mean response to a concentration of NE originally equipotent with neurogenic stimulation was 67 ± 17% of control values (n = 7).

Electrical TNS of the external jugular vein (Table 1), as well as portions of the facial vein proximal to those used in the present study (unpublished observations), never elicited relaxation. The single-phase contractile response to TNS at 10 Hz was 25% of the maximum NE response for the control tissue, and 28% in the presence of propranolol (10⁻⁶ M). When the vein segments selected for study were taken close to the buccal segment, a composite of relaxation and contraction could be observed.

The relationship between stimulus frequency and steady state relaxation response was determined (Fig. 4). There was no significant difference in the responses at 8 and 16 Hz. A response of every tissue at 0.5 Hz was observed and was approximately 10% of maximum dilation.

**RESPONSE TO AGONISTS**

**Norepinephrine**

On addition of NE to the bath, the external jugular and proximal segments of the facial veins contracted. The ED₉₀ for the external jugular vein was 0.012 μM (Fig. 5). In contrast, the segments of the facial vein used in this study relaxed on addition of NE to the bath. This relaxation to NE was not well maintained in most of the preparations. Attempts to minimize this phenomenon by prolonging the resting period between sequential exposures to NE, by the use of rapid cumulative additions of the drug, or by reducing the number of individual drug additions to a minimum were ineffective. Consequently, the control dose-response curve to NE on the buccal segment of the facial vein (Fig. 6) consists of mean responses from 18 preparations. Each preparation was used for two different responses prior to obtaining a maximum relaxation. The ED₉₀ for NE obtained in this manner was 0.114 μM (Fig. 6). In the presence of phenoxybenzamine (10⁻⁶ M) no difficulty was encountered in obtaining a maintained NE-induced relaxation. Under these circumstances the

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<th>Response to transmural nerve stimulation* as % of the maximum response to l-norepinephrine</th>
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<tr>
<td></td>
<td>Anterior facial</td>
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<tr>
<td>Control, phase A</td>
<td>46.4 ± 2.72 (12)†</td>
</tr>
<tr>
<td>Control, phase B</td>
<td>46.4 ± 3.35 (15)†</td>
</tr>
<tr>
<td>Phenoxybenzamine (10⁻⁶ M)</td>
<td>91.8 ± 6.2 (6)†</td>
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<tr>
<td>Propranolol (10⁻⁶ M)</td>
<td>47.1 ± 5.9 (6)†</td>
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Results are expressed as mean ± se. Numbers in parentheses denote number of observations.

* Transmural nerve stimulation (supramaximal voltage, frequency 16 Hz, pulse duration 0.3 msec).

† Relaxation.
ED₉₀ for NE was 0.031 μM, and indicated a 3.7-fold shift in the dose-response curve to the left (Fig. 6).

The β-adrenergic blocking agent, propranolol (10⁻⁶ M), reversed the NE-induced relaxation. The ED₉₀ for this presumably α effect of NE (since it was blocked by phenoxybenzamine, 10⁻⁵ M) was 0.10 μM, very similar to that determined for the β-response (0.114 μM) (Fig. 5). The ED₉₀ for NE for the exclusively contractile response of the external jugular vein was 0.095 μM in the presence of propranolol.

To demonstrate that the NE-reversing effect of propranolol was due to its β-receptor blocking action, the effect of this blocking agent on the relaxation to papaverine (3-6 × 10⁻⁷ M) was tested. The mean relaxation to papaverine after propranolol, expressed as a percentage of that before, was 101 ± 14.5 (n = 7).

**Histamine**

To test the response of the buccal segment of the facial vein to nonadrenergic stimuli, dose-response curves for histamine also were obtained. The addition of histamine to this segment of the vein resulted in contractile responses, the ED₉₀ being 0.345 μM (Fig. 5).

**UPTAKE OF ³H-NE AND ¹⁴C-SUCROSE**

The uptake of ³H-NE into the facial vein was separated into three components. The uptake into the extracellular space was assumed to be equivalent to that of the ¹⁴C-sucrose, namely, 0.39 ± 0.03 ml/g of tissue (n = 7). The extracellular extraneuronal (cocaine-insensitive) uptake of ³H-NE was 4.68 ± 0.57 ml/g of tissue (n = 7). The neuronal (cocaine-sensitive) uptake capacity was 27.30 ± 5.04 ml/g of tissue (n = 7).

**ENZYME ACTIVITY**

MAO and COMT activity were determined for the relaxing segments of facial veins taken from seven rabbits. The MAO activity (3.8 ± 1.1 μmol/g of protein per hour) and the COMT activity (19.6 ± 5.2 nmol/g of protein per hour) were in the same range as values previously obtained for a number of veins. The aorta was used as a control tissue in these determinations and its MAO activity was 2.3 ± 0.26 μmol/g of protein per hour and COMT activity was 20.8 ± 4.0 nmol/g of protein per hour.

**Discussion**

Characteristically NE elicits a contractile response of vascular tissue which can be blocked by α-adrenergic blocking agents such as phenoxybenzamine. The pres-
ence of \(\beta\)-receptors mediating relaxation usually is observed in isolated vascular tissue only when the tissue is actively contracted. Thus the present observation that the immediate response of the buccal segment of the facial vein to adrenergic stimuli (NE, isoproterenol, TNS) is relaxation is unusual among vascular tissues and implies that this tissue maintains intrinsic tone in the tissue bath. Prior incubation of the tissue with propranolol, a \(\beta\)-adrenergic receptor blocking agent, resulted in a contractile response to NE or TNS and shifted the dose-response curve of isoproterenol to the right. Thus it appears likely that the biphasic relaxation is a consequence of \(\beta\)-adrenergic receptor activation.

Light microscopic examination of the vein, although showing some modestly unusual features, demonstrated a predominantly circularly orientated smooth muscle in the media. No exclusively longitudinal smooth muscle layer was seen. Thus the relaxation observed in the vessel rings is not due to contraction of muscle orientated in a longitudi- nal axis which can sometimes give misleading results in experimentation in vitro.13

Fluorescence microscopy for catecholamines revealed a vessel with a heavy adrenergic innervation which was distributed throughout the entire thickness of the media. Medial innervation has been reported but only in the outer one-third to one-half of the blood vessel wall.14 This pattern of innervation, however, was not restricted to the relaxing segment but extended a considerable distance on either side of it. The heavy density of the adrenergic innervation is reflected by the high value for the neuronal uptake of \(^{3}H\)-NE, which is among the higher values found in veins.5 Because the neurons present just subjacent to the outer and inner vein surfaces would take up \(^{3}H\)-NE and tend to prevent its access to the deeper layers of the media, this value is not a good reflection of the nerve density in this vessel. The pattern of the frequency-response curve is similar to that obtained for other vessels with a medial innervation (the short saphenous vein and the proximal saphenous artery) in that the response at 8 Hz is similar to that at 16 Hz and significant contractile responses occur at 0.5 Hz. This contrasts with vessels in which the innervation is limited to the adventitialmedial junction when detectable responses are seen only at 1 Hz and the response at 16 Hz is much greater than that at 8 Hz.15

The biphasic contractile response of the rabbit ear artery is thought to be the consequence of a combination of an initial propagated contraction followed by a slower nonpropagated response.13, 15, 16, 17 In the present experiments a similar pattern of relaxation was observed. If the hypothesis is correct and can be extended to the relaxation in this vessel, it would suggest that active relaxation can be propagated through the vessel wall if it is elicited in the presence of preexisting intrinsic tone. The electrophysiological correlates of this response are entirely unknown but would be of considerable interest.

The adrenergically mediated relaxation could be elicited only within a rather restricted area (Fig. 1). Immediately proximal to the area the response usually was an initial rapid relaxation followed by contraction. At a greater distance the response was exclusively contraction. Relaxation responses to NE in preparations taken from the buccal segment disappeared quickly with repeated addition of the drug. They were not replaced by a contractile response, even in the presence of very high concentrations of NE. Blockade of either the \(\alpha\) or \(\beta\)-adrenergic receptors resulted in a preparation which responded reasonably consistently throughout the course of a normal experiment. It seems unlikely that any of the known pharmacological effects of these blocking agents would account for the maintained response of the tissue to NE after their addition to the tissue bath.

The underlying reason for this unusual response of the facial vein to adrenergic stimuli is unknown. In comparing this blood vessel with other vein segments of the vascular tree, the only outstanding difference appears to be the biphasic relaxation to both TNS and NE. The neuronal and extraneuronal uptakes of \(^{3}H\)-NE correspond to the observed innervation and compare favorably with findings for other vascular tissues.8 The metabolizing enzymes MAO and COMT were both present in the facial vein and their activities were within the range reported for a series of other veins.8

The ED\(_{50}\) for the relaxant effect of NE was 0.114 \(\mu\)M, while in the presence of propranolol the ED\(_{50}\) for the \(\alpha\) effect of NE was 0.10 \(\mu\)M. There was no significant difference between these two values. However, in the presence of phenoxybenzamine there was a 3.6-fold shift in the relaxation dose-response curve for NE. Part of this shift must result from the ability of phenoxybenzamine to block neuronal uptake of NE, because the results indicate that relaxation is a \(\beta\)-adrenergic receptor-mediated response.

These results are consistent with the hypothesis that the segment of the facial vein opposite the mouth cavity contains a predominant number of \(\beta\)-adrenergic receptors that mediate relaxation. Exposure to exogenous NE or to NE released from the heavy plexus distributed throughout the media results in an overwhelming \(\beta\)-mediated response. This response is increased in the presence of an \(\alpha\)-adrenergic blocking agent. After \(\beta\)-receptor blockade by propranolol the response is one of contraction mediated through \(\alpha\)-receptors. This reversal is due to the \(\beta\)-receptor blocking effects of this drug. The physiological or teleological reason for this peculiarity can only be speculated. The dilating segment appears to be opposite the rabbit cheek wall and this is a potentially expandable structure. If, during sympathetic activity, when presumably intense facial venaconstriction can occur, the cheek pouch was distended, blood flow might cease or the vein might suffer damage. However, the dilation of that part of the vein in the cheek under these circumstances, although making little difference to total blood flow, would tend to preserve the lumen and perhaps the integrity of the vessel.

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Uptake of Infarct-Imaging Agents in Reversibly and Irreversibly Injured Myocardium in Cultured Fetal Mouse Heart

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SUMMARY We studied the specificity of uptake of infarct-imaging agents for reversibly or irreversibly injured myocardium independently of blood flow by using intact beating fetal mouse hearts in organ culture. Reversible injury resulted from deprivation of oxygen and glucose for 4 hours at 37°C; irreversible injury, from similar deprivation at 42°C. At the end of the insult, uptake of 99mTc(Sn)-labeled pyrophosphate, glucoheptonate, or tetracycline was markedly increased in irreversibly damaged and, to a lesser degree, in reversibly injured hearts. After 24 hours of recovery, necrotic hearts accumulated even more pyrophosphate and tetracycline but less glucoheptonate. Uptake of radiolabeled tetracycline increased only in irreversibly injured hearts. Pyrophosphate uptake was not reduced in hearts cultured in calcium-free medium. These findings suggest that 99mTc(Sn)-labeled pyrophosphate, tetracycline, and glucoheptonate preferentially localize in irreversibly damaged myocardium; the 99mTc(Sn) complex modifies the specificity of uptake; and the uptake of 99mTc(Sn)-pyrophosphate appears unrelated to calcium uptake.

MANY radiopharmaceuticals have been used to visualize acute myocardial infarction scintigraphically, but little is known about the nature of uptake into irreversibly and reversibly damaged myocardium. Estimation of the extent of infarction and discrimination between irreversibly damaged myocardium and potentially salvageable myocardium is obviously of great clinical importance.

It appears that the distribution of these radiopharmaceuticals in infarcted myocardium is not only related to the severity of cell injury, but also to the degree of remaining blood flow. Therefore, studies in conventional animal models employing acute experimental coronary occlusion are often not conclusive, since regions with permanent cell damage overlap with regions containing reversibly or marginally injured cells. Thus, an experimental model which exhibits more uniform cell injury and in which uptake is independent of blood flow would be more useful to evaluate the uptake of these infarct-imaging agents.

The intact beating fetal mouse heart in organ culture has been found useful for studying the biochemical and ultrastructural responses of myocardial cells to oxygen and glucose deprivation, two aspects important in ischemia. In this model, the severity of cell injury can be controlled: if the deprivation occurs at 37°C the injury is reversible, but at 42°C necrosis occurs. Moreover, the hearts can be maintained in organ culture for days and therefore can be studied at various times during recovery from the insult. Accordingly, this experimental model was used to study the uptake of infarct-imaging agents both in relation to the severity of cell injury and at various times during recovery from insult. In addition, since the infarct-imaging agents are supplied to the hearts via a static reservoir, uptake was studied independently of blood flow.
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