Br. J. Pharmac. (1985), **84**, 265–271

An electrophysiological study of α-adrenoceptor mediated excitation-contraction coupling in the smooth muscle cells of the rat saphenous vein

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1 The effects of perivascular nerve stimulation and application of exogenous α-adrenoceptor agonists on the rat saphenous vein were studied by simultaneous recordings of electrical and mechanical activities.

2 The resting membrane potential of the saphenous vein averaged −65 mV. Perivascular nerve stimulation elicited excitatory junction potentials (e.j.ps) and slow depolarizations. Contraction was observed when either the e.j.p. or the slow depolarization reached a critical threshold of about −55 mV.

3 Exogenously applied noradrenaline, B-HT 920 and clonidine induced depolarization and contraction similar to the slow depolarization. The responses to these agonists and the slow depolarizations were antagonized by yohimbine, but not by prazosin.

4 The selective α_1-adrenoceptor agonists phenylephrine and methoxamine had very little effect on the electrical and mechanical activities of the saphenous vein.

5 It was concluded that in the rat saphenous vein, only α_2-adrenoceptors are present and that these receptors mediate the slow depolarization and contraction induced by nerve stimulation.

Introduction

The α-adrenoceptors in vascular smooth muscle have been subdivided into two main classes: α_1- and α_2-adrenoceptors. The properties of α_2-adrenoceptors are different from α_1-adrenoceptors. Thus, in the pithed rat, the pressor effect of α_2-adrenoceptor agonists is about 30–40 mmHg lower than that produced by α_1-adrenoceptor agonists (Timmermans *et al.*, 1983). Similar responses were obtained by tension studies on the dog isolated saphenous vein (Cavero *et al.*, 1983). The pressor or contractile responses due to the activation of the α_2-adrenoceptor are dependent on extracellular Ca and can be inhibited by Ca-antagonists (Van Meel *et al.*, 1981; Godfraind *et al.*, 1982; Cavero *et al.*, 1983; Timmermans *et al.*, 1983). In general, the α_1-adrenoceptor mediated responses are less dependent on external Ca and not as susceptible to the action of Ca-antagonists (Godfraind *et al.*, 1982; Cavaro *et al.*, 1983; Timmermans et al., 1983).

From electrophysiological studies, there is evidence that only α_2-adrenoceptors are present in the guinea-pig renal vein (Makita, 1983) and the dog mesenteric vein (Suzuki, 1984). The α_2-adrenoceptors are functionally innervated in the dog mesenteric vein (Suzuki, 1984). In the present study, we investigated the neuromuscular transmission in the rat saphenous vein. We further classified the type of α-adrenoceptors present by studying the effects of selective α_1- and α_2-adrenoceptor agonists and antagonists. By simultaneous recording of cellular electrical activity and contraction of the vein, we were also able to study the excitation-contraction process involved after stimulation of the adrenoceptors.

Methods

Ring segments of saphenous veins 3 mm long from male Wistar rats (250–350 g) were used in the experiments. The experimental set-up and procedures were similar to those previously described (Cheung, 1984). Tension was measured by two fine tungsten wires inserted through lumen of the vein. A resting tension of 50–100 mg was applied. One of the tungsten wires through the lumen and another wire running parallel to the preparation were used as

stimulating electrodes. The temperature of the preparations was maintained at 36–37°C by continuous superfusion with physiological solutions (Cheung, 1982) containing 1 μM propranolol at a rate of 4 ml min^{-1}. Application of α-adrenoceptor agonists to the preparations was made by injection of single bolus doses of the drug to the bath. This method of application of drugs allowed the temporal relationship between the electrical and mechanical activities to be studied (Cheung, 1984). Fibre-filled glass micropipettes (Omega Dot) of 40–60 MΩ resistance filled with 3 M KCl were used in the experiments.

Results

Effect of perivascular nerve stimulation

The resting membrane potential of the saphenous vein was -65 ± 0.6 mV ($n = 28$ preparations). Spontaneous depolarizations of the membrane similar to those in the guinea-pig mesenteric vein (Suzuki, 1981) were observed occasionally (Figures 2d and 4d). These spontaneous electrical activities were not blocked by prazosin or yohimbine (Figure 2d). Stimulation of the vascular smooth muscle cells

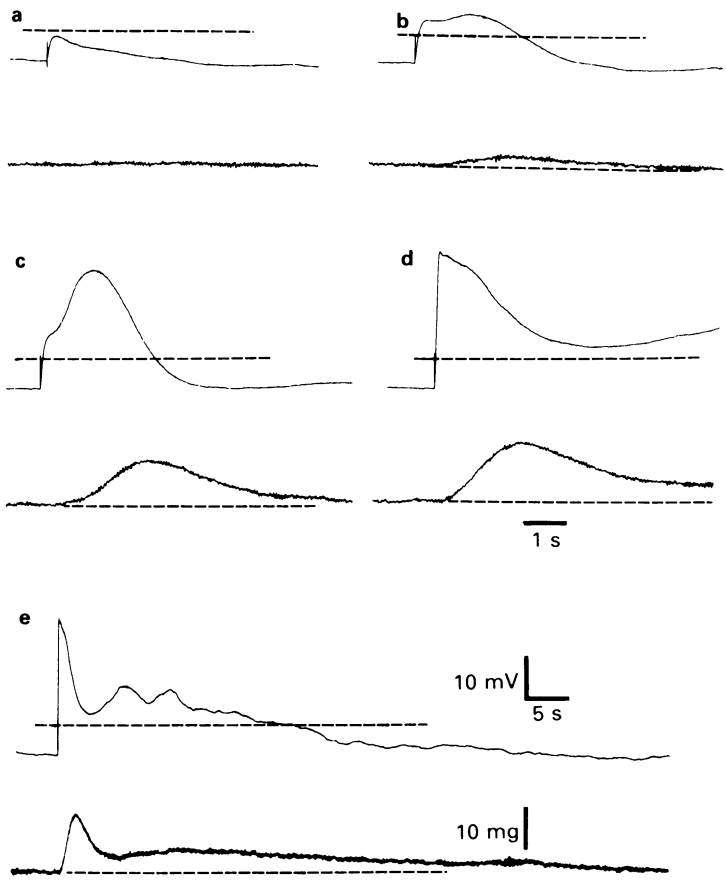

Figure 1 Effect of increasing stimulus intensity on the electrical (top traces) and mechanical (lower traces) activities of the rat saphenous vein. (a) Stimulation of the perivascular nerves with a single pulse (65 V, 0.1 ms duration) elicited an e.j.p. and no contraction. (b) Increasing the stimulus intensity to 68 V elicited an increase in amplitude in the e.j.p. and a small active response resulting in a small contraction. The active response and the contraction increased with increasing stimulus intensity ((c), 70 V; (d), 80 V). Following the e.j.p., a second component — the slow depolarization, with a longer duration of action could be identified (e). Note change of time scale in (e). Resting membrane potential of cell was −62 mV. The dotted lines mark −55 mV of the membrane potential and zero baseline of the resting tension.

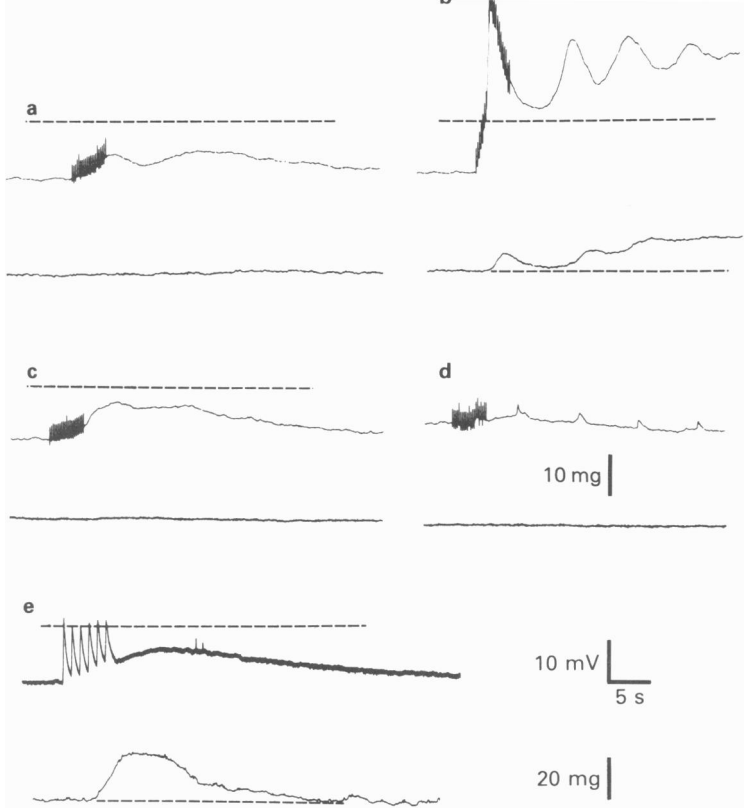

Figure 2 Effect of repetitive stimulation of the perivascular nerves on the electrical and mechanical activities of the rat saphenous vein (a–d) and rat tail artery (e). (a) Repetitive stimulation at 60 V, 0.1 ms duration at 5 Hz elicited a slow depolarization in the vein. (b) Increasing the stimulus strength to 80 V resulted in an increase in amplitude and oscillatory activities. While no change in tension was observed with the smaller depolarization in (a), the tension increase in (b) was associated with the membrane potential change. The slow depolarization was inhibited by yohimbine (d); (c) = control. (e) In the rat tail artery, a similar size slow depolarization to that of the saphenous vein in (a) and (c) was associated with a large contraction. The contraction in the tail artery also preceded the membrane potential change. Note an e.j.p. was elicited with each stimulus (1 Hz). The resting membrane potentials of the veins and the tail artery were all at − 68 mV. The dotted lines mark − 55 mV of the membrane potential and zero baseline of the resting tension.

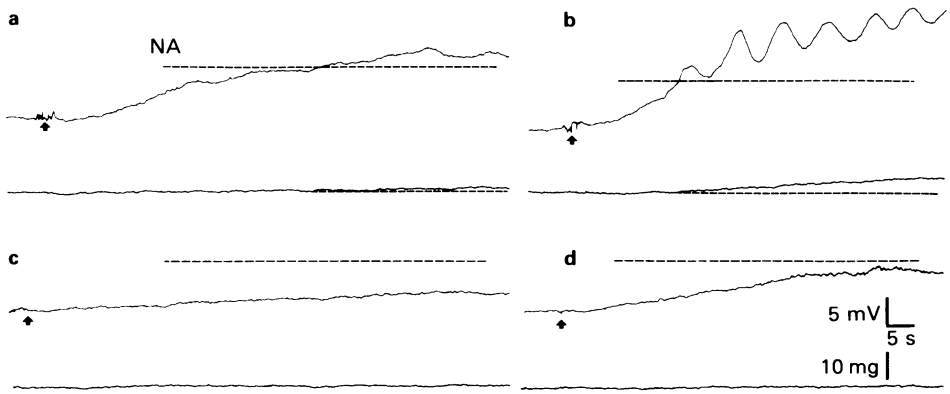

Figure 3 Effect of exogenous noradrenaline (NA) on the electrical and mechanical activities of the rat saphenous vein. (a) Application of noradrenaline (7×10^{-8} M) to the bath resulted in membrane depolarization and a small increase in tension when the membrane depolarized above − 55 mV. (b) A higher concentration of noradrenaline (1×10^{-7} M) caused oscillatory activities as well as the depolarization. An increase in tension was also observed. The effects of noradrenaline (7×10^{-8} M (c); 1×10^{-7} M (d)) were suppressed by yohimbine (2×10^{-7} M). The resting membrane potential of the preparation was − 64 mV. The dotted lines mark − 55 mV of the membrane potential and zero baseline of the resting tension.

either by nerves (Figure 2d) or by exogenous agonists (Figure 4d) enhanced these spontaneous activities. Stimulation of the perivascular nerves elicited two types of responses — the excitatory junction potential (e.j.p.) and the slow depolarization (Figure 1). The e.j.p. (Figure 1a) was not associated with contraction. With increasing stimulus intensity, a secondary active response was triggered (Figure 1b, c and d). Contraction was observed when the depolarization reached approximately −55 mV. These responses were not blocked by prazosin or yohimbine at 0.1 μM. Stimulation of the perivascular nerve also elicited a slow depolarization (Figure 1e). The e.j.p., the slow depolarization and the contraction were abolished by tetrodotoxin (TTX; 0.1 μM).

In most preparations, the slow depolarization could be selectively triggered using low stimulus strength and by repetitive stimulation (Figure 2). The slow depolarization showed oscillatory activity when depolarized beyond −60 mV (Figures 1e and 2b) and was antagonized by yohimbine (Figure 2d). Tension developed when the membrane reached the critical threshold of about −55 mV (Figure 2b), and the contraction was preceded by the membrane potential change, suggesting that the contraction was the result of membrane depolarization. This is in contrast to the rat tail artery where contraction occurred without the slow depolarization reaching any threshold and the contraction preceded the membrane potential change (Figure 2e; Cheung, 1984). The slow depolarization and the contraction in the tail artery were very sensitive to the α_1-antagonist prazosin (Cheung, 1984). From the neural responses, it appears that the receptors and the excitation-contraction coupling process in the rat saphenous vein are different from that of the rat tail artery.

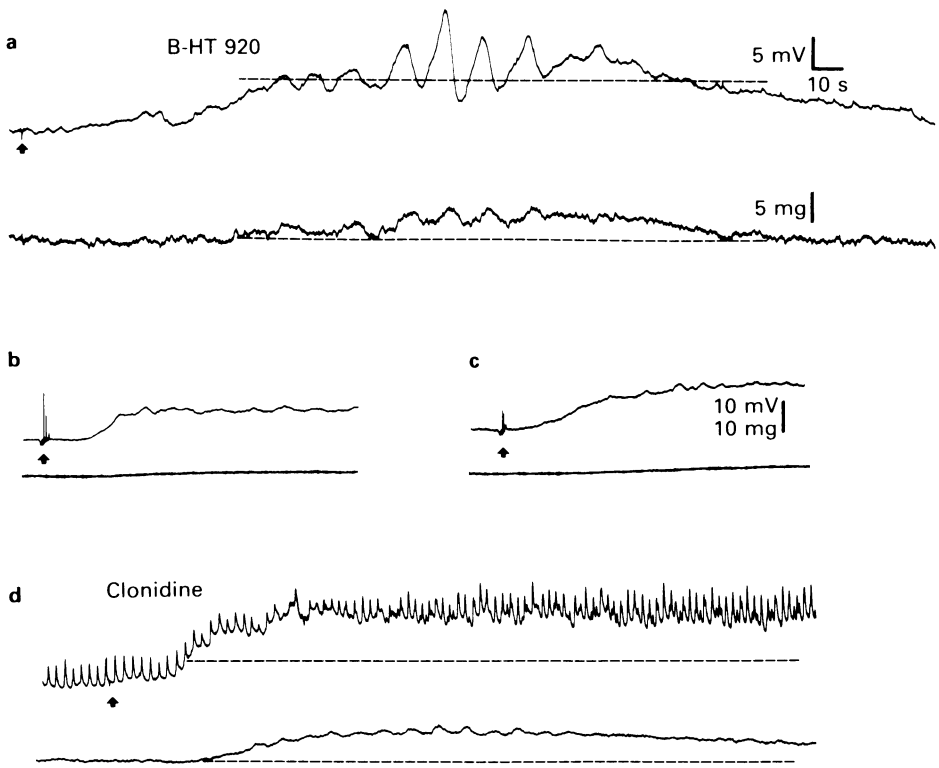

Figure 4 Effects of B-HT 920 and clonidine on the electrical and mechanical activities of the rat saphenous vein. (a) Application of B-HT 920 (7×10^{-6} M) resulted in membrane depolarization and oscillatory activities. Tension development associated with the membrane potential changes was observed. The effects of B-HT 920 were not antagonized by prazosin ((b) control; (c) after prazosin 2.6×10^{-7} M; B-HT 920 concentration was 5.5×10^{-6} M). (d) Clonidine (1.7×10^{-7} M) was also effective in generating electrical and mechanical responses similar to noradrenaline. Note the spontaneous electrical activity and the increase in frequency after clonidine. The dotted lines mark −55 mV of the membrane potential and zero baseline of the resting tension.

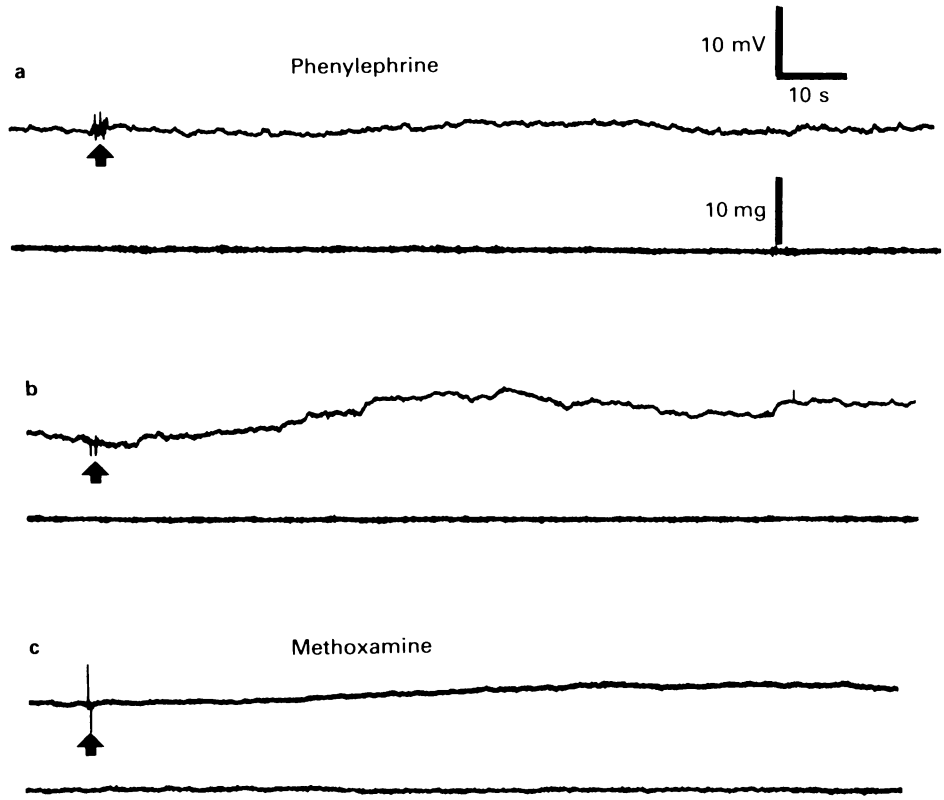

Figure 5 Effects of phenylephrine and methoxamine on the electrical and mechanical activities of the rat saphenous vein. (a) Phenylephrine (3.2×10^{-6} M) produced little changes in electrical or mechanical activities of the saphenous vein. (b) A higher concentration of phenylephrine (6.4×10^{-6} M) resulted in a small depolarization, but no contraction. Similarly, methoxamine up to 8×10^{-6} M (c) produced very little effect on the electrical and mechanical activities of the saphenous vein.

Effect of exogenous α-adrenoceptor agonists

Exogenous noradrenaline induced membrane depolarization and contraction was initiated when the threshold of −55 mV was reached (Figure 3a and b). The noradrenaline-induced depolarization also showed oscillatory activities and was antagonized by yohimbine (Figure 3).

The selective α_2-agonist B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo = [4,5-d] azepin-dihydrochloride) also induced membrane electrical and mechanical changes similar to those of noradrenaline (Figure 4). B-HT 920 induced responses by acting specifically on α_2-adrenoceptors and these responses were not antagonized by prazosin (2.6×10^{-7} M). Clonidine elicited similar responses to those of B-HT 920 (Figure 4d).

The selective α_1-agonists phenylephrine and methoxamine had very little effect on either electrical or contractile activities. At concentrations of phenylephrine and methoxamine ($> 10^{-6}$ M) that could almost maximally stimulate the α_1-adrenoceptors in the tail artery, no contraction in the vein and only a very slight depolarization of the membrane occurred (Figure 5).

Discussion

Stimulation of the perivascular nerves elicits two types of electrical responses in vascular smooth muscle cells — the e.j.p. and the slow depolarization (Cheung, 1982; 1984; Suzuki & Kou, 1983; Suzuki, 1984). The nature of the receptor mediating the e.j.p. is not well understood as it is not susceptible to blockade by α-adrenoceptor antagonists (Holman & Surprenant, 1979). In the rat tail artery (Cheung, 1984) and the rabbit ear artery (Suzuki & Kou, 1983), the slow depolarization is mediated by α_1-adrenoceptors and can be selectively blocked by prazosin. In the dog mesenteric vein, the slow de-

polarization is mediated by α_2-adrenoceptors and the response can be selectively blocked by yohimbine (Suzuki, 1984).

The present study demonstrates that the slow depolarization in the rat saphenous vein is also mediated by α_2-adrenoceptors. These adrenoceptors could be activated by exogenous noradrenaline and the selective α_2-adrenoceptor agonists B-HT 920 and clonidine. There appear to be no α_1-adrenoceptors in the rat saphenous vein as evidenced by the lack of effect of α_1-adrenoceptor agonists, phenyleprine and methoxamine, and the selective α_1-adrenoceptor antagonist prazosin. Also, only α_2-adrenoceptors are present in the guinea-pig renal vein (Makita, 1983).

Simultaneous recordings of membrane potential change and tension development of the rat saphenous vein indicate that contraction mediated by stimulation of the α_2-adrenoceptors is dependent on membrane depolarization to the critical threshold of approximately -55 mV. Presumably, further depolarization would open up the voltage-dependent channels to allow influx of extracellular Ca to initiate contraction (Bolton, 1979). From ^{45}Ca flux studies, the clonidine stimulated contraction in the rat aorta is shown to be totally dependent on extracellular calcium (Godfraind *et al.*, 1982). The increase in diastolic blood pressure in pithed rats and cats by selective stimulation of α_2-adrenoceptors is inhibited by Ca-antagonists (Cavero *et al.*, 1983; Timmermans *et al.*, 1983) and divalent metal ions (Van Meel *et al.*, 1981). These *in vivo* observations have been confirmed by *in vitro* experiments in the perfused hindquarters of rats (Van Meel *et al.*, 1983) and canine isolated saphenous vein strips (Cavero *et al.*, 1983). Thus, the excitation-contraction coupling process mediated by the α_2-adrenoceptors is similar to the action of high concentrations of KCl and could be classified as electro-mechanical coupling (Bolton, 1979; Cheung, 1984). The K-depolarization induced contraction is dependent on Ca influx through the voltage-dependent channels and is suppressed by Ca-antagonists (Van Breeman *et al.*, 1981). In fact, there is a highly significant linear relationship between the inhibitory activities of the Ca-antagonists on α_2-adrenoceptor mediated pressor responses in the pithed rats and the contraction of K-depolarized rabbit aorta strips (Van Meel *et al.*, 1983).

The contraction mediated by the α_1-adrenoceptors is less dependent on extracellular Ca and is generally less sensitive to inhibition by Ca-antagonists (Godfraind *et al.*, 1982; Cavero *et al.*, 1983; Timmermans *et al.*, 1983). In the rat tail artery, large contractions sensitive to blockade by prazosin could be elicited by endogenous and exogenous noradrenaline when the membrane potential was still below the critical threshold for electro-mechanical coupling. These observations suggest that while pharmaco-mechanical coupling is a very important feature of the α_1-adrenoceptor stimulated contractions (Cheung, 1984), electro-mechanical coupling is the sole mechanism underlying the contraction due to α_2-adrenoceptor stimulation.

This work was supported by a grant from the Ontario Heart Foundation. D.W.C. is a MRC scholar.

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(*Received June 22, 1984.*
Revised August 29, 1984.)