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SPECIAL REPORT

Diosmin-induced increase in sensitivity to Ca^{2+} of the smooth muscle contractile apparatus in the rat isolated femoral vein

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The effect of diosmin, a flavone derivative, on the Ca^{2+} sensitivity of the venous contractile apparatus was investigated in chemically (β-escin) skinned strips from the rat isolated femoral vein. Diosmin (0.5–10 μM) shifted to the left the concentration-response curve to Ca^{2+} (0.05–5 μM). The maximal effect was observed in the presence of 1 μM diosmin which increased the contractile response evoked by 0.15 μM Ca^{2+} from 26.3% to 78.9% of the maximal Ca^{2+}-induced response. This work demonstrates that the venotonic action of diosmin involves an increase in the Ca^{2+} sensitivity of the contractile apparatus. Such a mechanism of action could represent a new and important means of therapeutic control of vasomotor activity.

Keywords: Diosmin; Ca^{2+}sensitivity; contractile apparatus; skinned fibres; β-escin; vascular smooth muscle; femoral vein

Introduction Modulation of vascular smooth muscle tone is clinically relevant in both systemic arterial and venous vascular beds. A currently prescribed drug in venous insufficiency is the hemisynthetic diosmin (Diovenor) which modifies the *in vitro* metabolism of noradrenaline by the varicose human saphenous vein (Araujo *et al.*, 1991). Diosmin ((7[6-deoxy-α-L-mannopyranosyl-β-D-glucocopyranosyl] oxyl]-5-hydroxy-2(3-hydroxy-4-methoxyphenyl)-4-*H*-1-benzopyran-4-one) is a flavone derivative exhibiting a high venoselectivity (Dacquet *et al.*, 1992). However, its mechanism of action remains largely unknown.

Recently, it has become clear that smooth muscle tone can be modulated at the level of the Ca^{2+} sensitivity of the contractile apparatus (Nishimura *et al.*, 1988; Kitazawa *et al.*, 1991). Whether or not drugs administered in vascular diseases act directly at the site of the contractile apparatus remains poorly investigated. *In vitro* studies have suggested the existence of an action produced by diosmin on calcium-dependent contractility (Dacquet & Finet, 1990; Dacquet *et al.*, 1992). We thus designed the present work to examine the effect of diosmin on the Ca^{2+} sensitivity of the smooth muscle contractile apparatus in β-escin skinned venous strips.

Methods Experiments were performed in the rat isolated femoral vein. Animals were killed with an overdose of pentobarbitone (40 mg kg^{-1}). Strips (200–300 μm in diameter, 4–5 mm in length) were cut transversely from veins in which connective tissue and endothelium were removed. Contraction was measured isometrically. Strips were chemically skinned with β-escin (80 μM for 30 min). Solutions as well as procedures used to skin smooth muscle strips and to impair the functioning of the sarcoplasmic reticulum have been described in detail elsewhere (Savineau *et al.*, 1993; Savineau & Marthan, 1994). Experiments were performed at 25°C. Concentration-response curves to Ca^{2+} (0.01–5 μM) were constructed in a cumulative manner in either the absence or the presence of diosmin (0.1–10 μM) applied 30 min before and throughout the $[Ca^{2+}]$-tension curve. The $[Ca^{2+}]$-tension relationship was fitted using the Hill equation: $T/T_{max} = K[Ca^{2+}]^h/1 + [Ca^{2+}]^h$ where T is a fraction of the maximal Ca^{2+}-activated force in the absence of diosmin (T_{max}); K is a constant related to the $[Ca^{2+}]$ value corresponding to 50% of T_{max} (EC_{50}); h is the Hill coefficient.

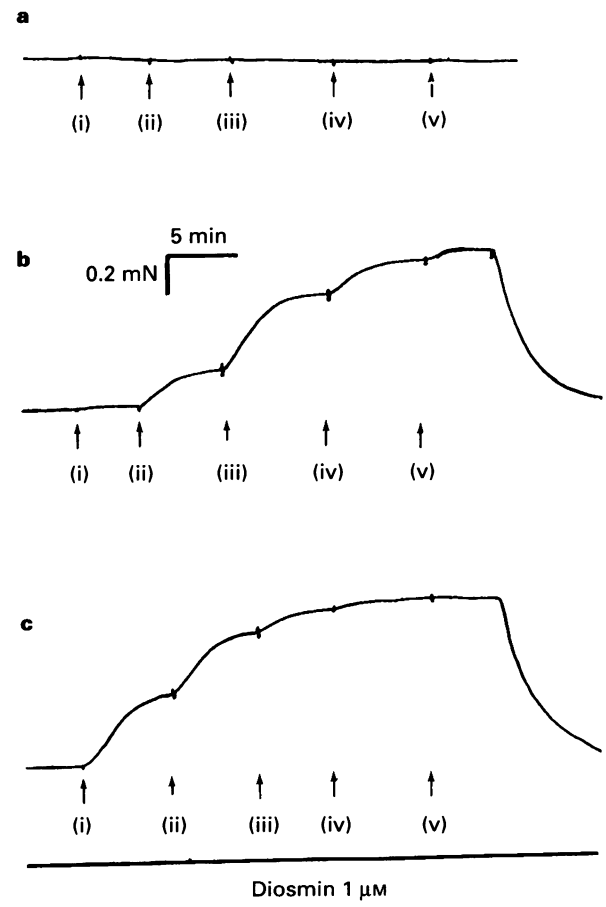

Figure 1 Effect of diosmin on Ca^{2+}-induced contractions in chemically skinned strips from the rat isolated femoral vein. (a) In the absence of β-escin treatment, Ca^{2+} concentrations of 0.08 (i); 0.15 (ii); 0.25 (iii); 0.76 (iv) and 1.75 μM (v) did not induce a contractile response. Prior to the application of Ca^{2+}, the strip was bathed for 10 min in the presence of a Ca^{2+}-free, 0.5 mM EGTA solution. (b) After β-escin treatment (80 μM for 30 min), the same Ca^{2+}-concentrations (i) to (v) induced concentration-dependent and maintained contractions. (c) In the presence of diosmin (1 μM), contractions induced by Ca^{2+} (0.08 to 0.76 μM) were increased. In (b) and (c) at completion of the Ca^{2+}-tension curve, relaxation was obtained by reperfusing the strip with a Ca^{2+}-free solution containing 10 mM EGTA.

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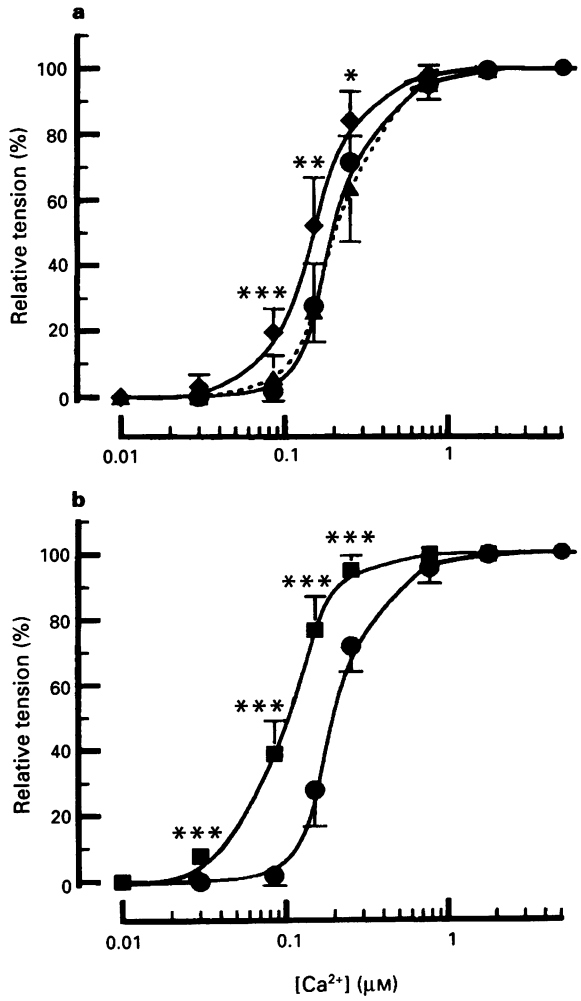

Figure 2 Effect of diosmin on the $[Ca^{2+}]$-tension relationship of the contractile apparatus in the rat isolated femoral vein. Concentration-response curves for the effect of Ca^{2+} were obtained in the absence (●, a, b) and in the presence of diosmin 0.1 μM (▲, a); 0.5 μM (◆, a) and 1 μM (■, b). The abscissae indicate the micromolar concentration of Ca^{2+} on a log scale. The ordinates indicate the tension expressed as a percentage of the maximal Ca^{2+}-induced contraction. Data points are means, $n = 10$ in the absence (●), and $n = 5$ in the presence (▲, ◆, ■) of diosmin. Vertical bars show s.d. $*P<0.05$; $**P<0.01$ and $***P<0.0001$ indicate a response in the presence of diosmin which is significantly different from the corresponding response in the control experiment.

The amplitude of contraction is expressed as mean ± s.d. for n, number of experiments. Significance was assessed by the Student's t test. A difference between means was considered significant when $P<0.05$.

Chemicals and drugs Chemicals were purchased from Sigma (St Quentin Fallavier, France). Hemisynthetic diosmin (Diovenor) was a kind gift of Laboratoire Innothéra (Arcueil, France). Diosmin was dissolved in dimethylsulphoxide (DMSO). The maximal concentration of DMSO in the solution was <0.1% and had no effect on the contractility of femoral venous strips.

Results Ca^{2+} (0.08–1.75 μM) did not produce a contraction in intact strips (Figure 1a) whereas, after β-escin treatment, Ca^{2+} induced concentration-dependent and maintained contractions (Figure 1b). Addition of 1 μM diosmin in skinned strips increased the amplitude of contractions induced by 0.02 to 0.76 μM Ca^{2+} (Figure 1c). For example, the contractile response evoked by 0.15 μM Ca^{2+} increased from 26.3% to 78.9% of the maximal Ca^{2+}-induced response. The $[Ca^{2+}]$-tension relationship of the skinned femoral vein in the absence of diosmin is shown in Figure 2a (●). EC_{50} and h values were respectively equal to 0.21 μM and 3.58. Diosmin (0.5–10 μM) shifted to the left the $[Ca^{2+}]$-tension relationship. The maximal effect was observed in the presence of 1 μM diosmin (Figure 2b). The EC_{50} value decreased from 0.21 in the absence of diosmin to 0.15 and to 0.10 μM in the presence of 0.5 and 1 μM diosmin, respectively. A higher concentration of diosmin had no further effect since the EC_{50} value was 0.16 μM in the presence of 10 μM diosmin (not shown). The Hill coefficient was only slightly reduced from 3.58 in the absence, to 2.83 in the presence of diosmin (1 μM) (Figure 2b).

Discussion To the best of our knowledge, this report is the first demonstration of a direct modulation of the Ca^{2+} sensitivity of the contractile apparatus by a venotonic drug. Diosmin increases the sensitivity to Ca^{2+} of the contractile apparatus from the femoral vein. This effect was particularly pronounced at low Ca^{2+} concentrations (0.02 to 0.76 μM, Figure 1) as previously reported in the rabbit depolarized saphenous vein (Dacquet *et al.*, 1992). The effect of diosmin at the site of the contractile apparatus could explain, at least in part, its venotonic action. A similar increase in the Ca^{2+} sensitivity of vascular smooth muscle has been observed in the presence of either phosphatase inhibitors (Gong *et al.*, 1992) or α-adrenoceptor agonists (Nishimura *et al.*, 1988). In the latter case, this increase appeared mediated by G-proteins and involved an enlarged phosphorylation of myosin light chains (Kitazawa *et al.*, 1991). Since both phosphatase and kinase activities control the tension state in vascular smooth muscle, precise investigation of the molecular target of diosmin require further biochemical investigations.

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