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***Urtica dioica L*. or Nettle (Urticaceae) is widely used in oriental Morocco to treat hypertension. Aqueous extract of Nettle (AEN) also exerts a hypotensive action in the rat *in vivo*. The aim of this work was to characterize the specific cardiac and vascular effects of AEN. In the isolated *Langendorff* perfused rat heart, AEN (1 and 2 g/l) markedly decreased heart rate and increased left ventricular pressure. Higher concentration (5 g/l) even led to cardiac arrest. Although carbachol mimicked the bradycardiac effect of AEN, atropine (a muscarinic receptor antagonist, 1 • M) did not modify the response. Beside its action on myocardium, AEN also affected vascular contractility. Indeed, AEN (0.1–5 g/l) produced a dose-dependent increase in basal tone of isolated rat aorta. This effect was endothelium independent and was abolished by 1 • M prazosin (an • 1-adrenergic antagonist). AEN had little additional effects when the aorta was precontracted by noradrenaline (1 • M) or KCl (40 mM). Our data indicate that AEN produces a vasoconstriction of the aorta which is due to activation of • 1-adrenergic receptors. However, AEN also induces a strong bradycardia through non-cholinergic and non-adrenergic pathways which might compensate for its vascular effect and account for the hypotensive action of *Urtica dioica L* described *in vivo*.**

Keywords: phytotherapy; *Urtica dioica L*; Urticaceae; isolated perfused heart; thoracic aorta; rat.

INTRODUCTION

Urtica dioica L or nettle (Urticaceae) exerts different pharmacological effects, such as a stimulation of human lymphocyte proliferation (Wagner *et al.*, 1989) and an antiinflammatory action (Riehemann *et al.*, 1999), and is used in the treatment of prostatic hyperplasia (Lichius and Muth, 1997). In the pharmacopoeia, nettle is well known to be hypotensive (Garnier *et al.*, 1961) and hypoglycaemic (Newall *et al.*, 1996). In oriental Morocco, nettle appears to be one of the most used medicinal plants in the traditional therapy of arterial hypertension (Ziyyat *et al.*, 1997). Previous studies *in vivo* have shown that an extract of this plant produces a hypotensive action on normotensive cat and rat (Bromcamo *et al.*, 1983; Lasheras *et al.*, 1986). We previously confirmed this action and further showed the diuretic and natriuretic effects of *Urtica dioica L*. (Tahri *et al.*, 2000). The aim of this work was to obtain some insights into the mechanism(s) of action of this plant by studying the direct effects of an aqueous extract of nettle (AEN) on the contractility of isolated rat heart and aorta.

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MATERIAL AND METHODS

Preparation of aqueous extract. The plant was collected from Oujda city in oriental Morocco. The aqueous extract was obtained from the aerial part of the plant using a traditional method described in folk medicine: 10 g of plant was infused in 100 mL of boiled distilled water and incubated for 20 min. The aqueous extract was filtered and dried at 50•C.

Experiments on isolated perfused rat heart. Male Wistar rats 300–350 g were anaesthetized with urethane (2 g/kg, i.p.). The heart was rapidly excised and the aorta cannulated for *Langendorff* perfusion at 36•C using a constant flow of 12 mL/min with the following perfusate composition (in mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 1.5; $NaHCO_3$, 25; glucose, 10; pyruvate, 2; mannitol, 1.12. The perfusate was saturated with 95% O_2–5% CO_2 (pH = 7.4). A latex balloon was introduced into the left ventricle via the left atrium and connected to a pressure-transducer (Statham gauge Ohmeda, Biltho-ven, Holland) and paper recorder (Dash IV, Astro-Med, West Warrick, UK), for measurement of contractile parameters: heart rate (HR), left ventricular pressure (LVP). The rate pressure product (RPP = LVP • HR) was used as an index of contractility to evidence the global change in the contractile capacity of the myocardium. The balloon was progressively inflated to the maximal isovolumic conditions of work. The effect of AEN on the contractile parameters was studied after a 30 min equilibration period.

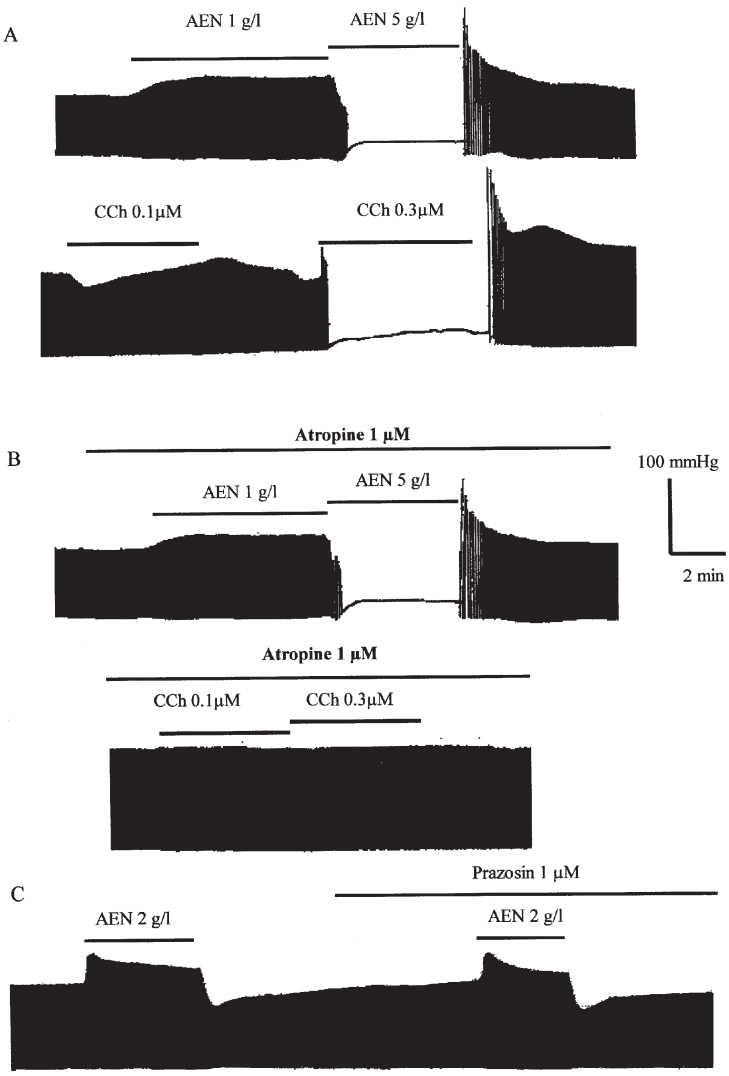

Figure 1. Original tracings showing the effect of aqueous extract of nettle (AEN) on left ventricular pressure of the isolated perfused rat heart. AEN was tested in control condition (*A, upper panel*) and in the presence of 1 μM atropine (*B, upper panel*) or 1 μM prazosin (*C*). The effects of AEN were compared with those of carbachol (CCh, 0.1 and 0.3 μM) under control condition (*A, lower panel*) and in the presence of atropine (*B, lower panel*)

Experiments on isolated rat thoracic aorta. Male Wistar rats weighing 250–300 g were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the thoracic aorta was removed and placed in Krebs–Henseleit solution (KHS). An aortic ring of about 2–3 mm in length was suspended between two stainless steel hooks in a 10 mL water-jacketed bath containing KHS of the following composition (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 1.6; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11. The tissue bath solution was maintained at 37 °C and gassed with 95% O_2–5% CO_2 (pH 7.4). The isometric contraction was recorded via a force-displacement transducer (Senso Nor, type 801) connected to a paper recorder (Leybold-Heraeus, type SE122). A tension of 1 g was initially applied to the ring which was equilibrated in the medium for 30 min. Before each experiment, vasoconstriction was initiated by 1 μM noradrenaline (N-Adr) in normal KHS. When a steady contraction was reached, 100 μM carbachol (CCh) was added to induce endothelium-dependent relaxation. This step was necessary to verify the integrity of the endothelium. To test the role of the endothelium in the effect of AEN, the endothelium was removed mechanically by gently rubbing off the lumen of the artery. The absence of CCh-induced relaxation was an indicator of successful endothelium denudation. AEN was tested either under basal conditions or on precontracted aorta by either N-Adr (1 μM) or elevated K^+ concentration (40 mM, equimolar replacement of NaCl with KCl). These experiments were performed on intact and denuded aorta in the absence and in the presence of prazosin (1 μM) in the incubation solution. In denuded aorta, relaxation was produced by 1 μM sodium nitroprusside (NPS) which produces endothelium-independent relaxation.

Chemicals. (-)-Norepinephrine hydrochloride (N-Adr), carbamylcholine chloride (CCh) and prazosin were purchased from Sigma Chemical, atropine from Labosi and sodium nitroprusside from Farco Chemical. All compounds were dissolved in water.

Statistics. The results are expressed as the mean ± SEM for n separate experiments. Data were analysed by Student's paired t-test. A difference was considered as statistically significant when $p < 0.05$.

RESULTS

Cardiac effects of AEN

In control isovolumic conditions, the left ventricular pressure (LVP) was 115 ± 12 mmHg, the heart rate (HR) was 177 ± 13 beats/min and the rate pressure product (RPP) was 19638 ± 1355 mmHg.beats/min ($n = 6$). Figure 1A (upper panel) shows a representative experiment testing the effect of the aqueous extract of nettle (AEN) on a spontaneously beating isolated perfused rat heart. At 1 g/L, AEN produced a clear positive inotropic effect. As shown in Table 1, the positive inotropic effect of AEN was associated with a marked decrease in HR without any significant change in RPP (a global estimate of heart

Table 1. Effects of aqueous extract of nettle (AEN) on left ventricular pressure (LVP), heart rate (HR) and rate pressure product (RPP) of the isolated perfused rat heart

		AEN		
	Control	1 g/L	2 g/L	5 g/L
LVP (mmHg)	115 ± 12	155 ± 19[a]	176 ± 18[a]	
HR (beats/min)	177 ± 13	133 ± 19[a]	88 ± 10[a]	Cardiac arrest
RPP (mmHg.beats/min) ×10^3	19.6 ± 1.3	19.6 ± 1.1	16.6 ± 1.7	

Values are mean ± SEM. Paired t-test

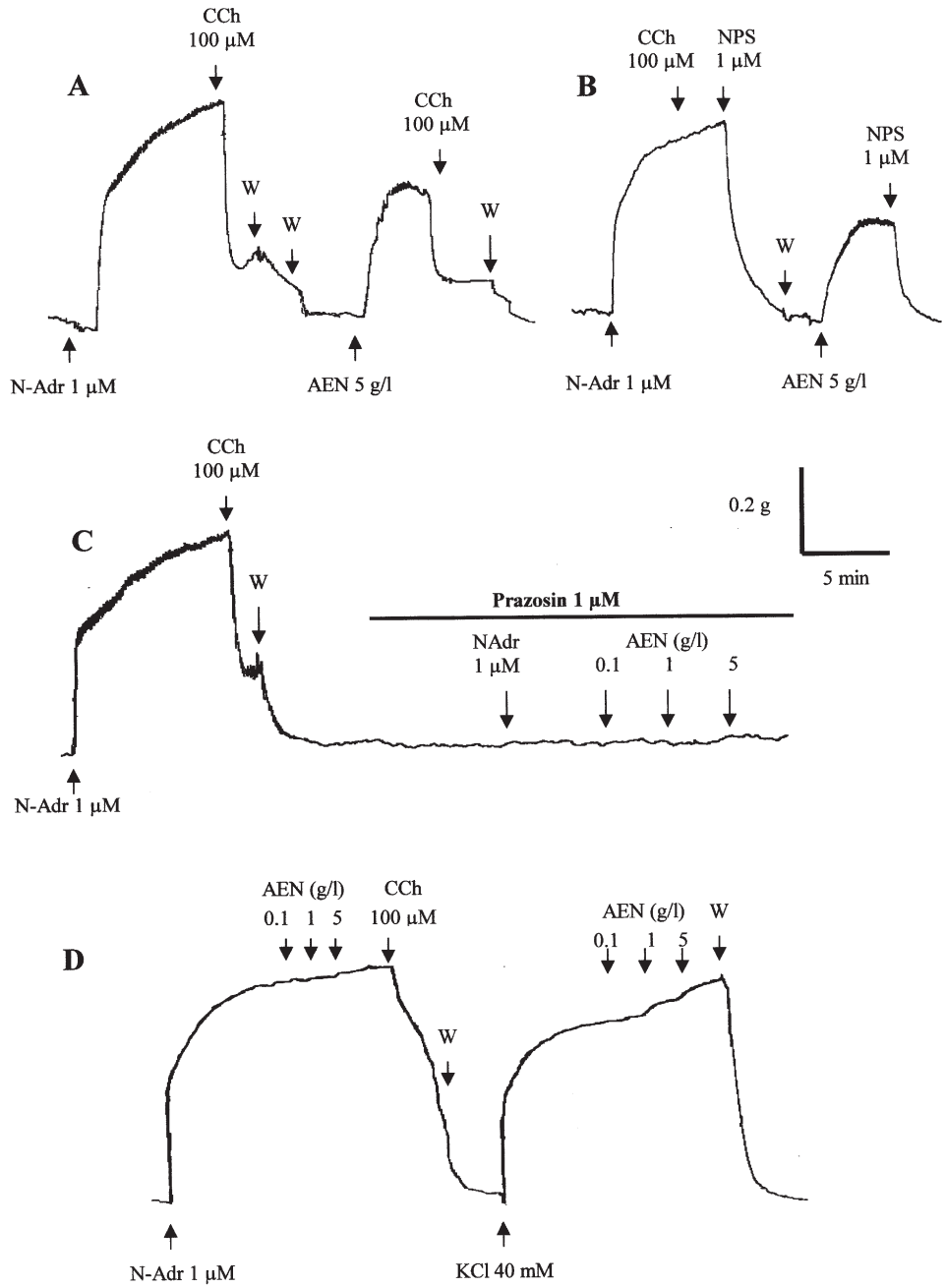

Figure 2. Original tracings showing the effect of aqueous extract of nettle (AEN) on intact isolated rat aorta. Each experiment started with a test of the response of the aorta to noradrenaline (N-Adr, 1 • M). Then the aorta was exposed either to carbachol (CCh, 100 • M), AEN (0.1 to 5 g/L) or sodium nitroprusside (NPS, 1 • M) as indicated by the arrows. W indicates the washout of the solution by fresh KHS. (*A*), (*C*) and (*D*): intact aorta; (*B*): denuded aorta. In (*D*), AEN and N-Adr were tested after application of 1 • M prazosin

work). Increasing the AEN concentration to 2 g/L induced a more pronounced negative chronotropic effect (Table 1) associated with a stronger positive inotropic effect (Fig. 1C and Table 1). When the concentration of AEN was further increased to 5 g/L, the heart stopped beating (Fig. 1A and Table 1). All the effects of AEN were fully reversible upon return to control conditions (Fig. 1A). These experiments suggest that AEN induces primarily a bradycardia which leads to an increased contractility due to the classical negative force staircase of the rat myocardium.

As shown in Fig. 1A (lower panel), the inotropic and chronotropic effects of AEN resemble those of carbachol (CCh), a muscarinic receptor agonist. In order to examine whether AEN activates muscarinic receptors, the effect of AEN was tested in the presence of atropine, a muscarinic receptor antagonist. As shown in Fig. 1B (upper panel), AEN (1 and 5 g/L) produced a clear response in the presence of 1 • M atropine although the response to CCh was totally suppressed (Fig. 1B, lower panel). On average, the effect of AEN (2 g/L) was not significantly different in the absence or presence of 1 • M atropine: HR decreased by • 35% • 16% and • 25% • 12%, respectively ($n = 4$), while LVP increased by • 41% • 4% and • 29% • 3%, respectively ($n = 4$). Since AEN produces an α1-adrenergic-like response in the vasculature (see below), AEN was also tested in the presence of prazosin, an α1-adrenergic receptor antagonist, to examine whether activation of these receptors was involved in the cardiac effect of AEN. As shown in the experiment of Fig. 1C, the inotropic effect of AEN was similar in the presence or absence of 1 • M prazosin ($n = 2$).

Table 2. Effect of aqueous extract of nettle (AEN) on basal tone of intact and denuded aorta and on tension developed by N-Adr (1 • M) or by KCl (40 mM)

	Initial tension (g)	AEN		
		0.1 g/L	1 g/L	5 g/L
		Tension (g)		
Intact aorta	0	0.02 • 0.01	0.10 • 0.03[a]	0.44 • 0.03[c]
Denuded aorta	0	0.01 • 0.01	0.06 • 0.02[a]	0.35 • 0.08[b]
Aorta precontracted by N-Adr	0.69 • 0.05	0.69 • 0.05	0.71 • 0.05[a]	0.73 • 0.07[a]
Aorta precontracted by KCl	0.53 • 0.04	0.54 • 0.04	0.57 • 0.03[b]	0.63 • 0.03[c]

Values are mean • SEM. Paired *t*-test
[a] $p < 0.05$;
[b] $p < 0.01$;
[c] $p < 0.001$ ($n = 6$) versus initial tension.

Vascular effects of AEN

The effects of AEN were then studied on the isolated rat thoracic aorta. Each experiment usually started with a test of the response of the aorta to noradrenaline (N-Adr), a well known vasoconstricting agent, and CCh, an endothelium-dependent relaxing agent. In an intact aorta (Fig. 2A), N-Adr (1 • M) induced a strong contraction which was antagonized by the application of CCh (100 • M). In a denuded aorta (Fig. 2B), the contracting effect of N-Adr was preserved but the relaxing effect of CCh was totally eliminated. As shown in Fig. 2A and B, AEN (5 g/L) produced a rise in the basal aortic tension which resembled that of N-Adr. Indeed, AEN induced a vasoconstriction both in intact (Fig. 2A) and denuded aorta (Fig. 2B). Besides, CCh antagonized the effect of AEN like that of N-Adr (Fig. 2A). Finally, in denuded aorta, the NO-donor sodium nitroprusside (NPS, 1 • M) antagonized both the vasoconstricting effects of N-Adr and AEN (Fig. 2B). As shown in Table 2, the vascular contractile response to AEN was dose dependent, with a threshold concentration of 1 g/L. The effect of AEN was independent of the presence of the endothelium since the same dose-response was obtained when AEN was tested on denuded aorta (Fig. 2B, Table 2).

The resemblance of the effects of AEN and N-Adr on the isolated rat aorta led us to examine the effect of AEN in the presence of the α1-adrenergic antagonist prazosin. As shown in Fig. 2C, prazosin (1 • M) totally blocked the effect of both N-Adr and AEN ($n = 3$). The α1-adrenergic effect of AEN was further confirmed by the little additivity of the effects of AEN and N-Adr. Indeed, as shown in Fig. 2D, when the aorta was precontracted by N-Adr (1 • M), AEN (0.1–5 g/L) produced a rather small additional effect (see Table 2 for statistics). Similarly, when precontraction was induced by raising the extracellular $K^{\bullet}$ concentration from 4.7 to 40 mM, AEN (0.1 to 5 g/L) had little additional effect (Fig. 2D and Table 2). Thus, when tested on the isolated aorta, AEN produced a vasoconstriction which was likely due to activation of α1-adrenergic receptors and a subsequent rise in intracellular Ca concentration.

DISCUSSION

Urtica dioica (Urticaceae, nettle) appears to be one of the most commonly used plants to treat arterial hypertension in oriental Morocco (Ziyyat *et al.*, 1997). Its hypotensive action has been shown in anaesthetized cat and rat (Bromcamo *et al.*, 1983; Lasheras *et al.*, 1986). In a previous study, we have shown that an intravenous perfusion of aqueous extract of nettle (AEN) induces diuretic and natriuretic effects on the anaesthetized rat (Tahri *et al.*, 2000). These effects could partly explain the decrease of the arterial pressure (AP) observed during the oral chronic treatment. However, an intravenous injection of AEN also provokes a fast decrease of AP associated with bradycardia (Lasheras *et al.*, 1986). The aim of this work was thus to elucidate the mechanisms of action of AEN. Both the myocardium and the vasculature could account for the fast decrease in AP observed *in vivo* and are therefore potential targets of AEN. We thus studied the effect of AEN in the isolated perfused heart and the isolated aorta, two biological preparations allowing the evaluation of direct effects on the organs without interference of nervous, hormonal and/or ionic homeostasis regulations.

The study performed on the isolated heart shows a dose-dependent bradycardia of AEN without intrinsic modification of contractile capacities which confirms the bradycardia observed *in vivo* by Bromcamo *et al.* (1983). This very fast and fleeting effect was similar to the action of CCh. Since nettle was shown to contain acetylcholine among its components (Adamski and Bieganska, 1984), it was obvious to question the implication of muscarinic receptors in the cardiac effect of AEN. However, the effect of AEN persisted in the presence of atropine (a muscarinic receptor antagonist) showing that it was independent of the cholinergic pathway. Bromcamo *et al.* (1983) proposed that the bradycardia observed *in vivo* could result from an inhibition of α-adrenergic receptors. However, this was clearly not the case since (1) the results in aorta show that AEN activates rather than inhibits α1-adrenergic receptors, and (2) prazosin neither mimicked nor modified the cardiac effects of AEN. This AEN-induced bradycardia would be responsible for the fast decrease of AP observed *in vivo* since it decreases the cardiac output which directly influences AP.

At the vascular level, a vasodilating effect of nettle has been reported in the pharmacopoeia (Garnier *et al.*, 1961). Besides, it is noteworthy that nettle also contains polyphenols and tannins (Chaurasia and Wichtl, 1987) which are well known for their vasorelaxant effects (Fitzpatrick *et al.*, 1995; Andriambeloson *et al.*, 1998; Middleton *et al.*, 2000). However, until now, the vasodilating action of nettle has not been experimentally established. Thus, in order to elucidate the vascular

effects of nettle, we tested the action of AEN on the rat isolated aorta. Surprisingly, we found that AEN provoked a dose-dependent increase in the basal contraction. Besides, AEN did not relax N-Adr- or KCl-precontracted aorta. According to these results, AEN exerts a vasoconstricting and not a vasorelaxing action. To examine whether this effect was a direct effect on the vascular smooth muscle or an indirect effect due to the release of endothelial contracting factors such as endothelin (Vanhoutte and Katusic, 1988), AEN was tested on denuded aorta. In these conditions, AEN still induced vasoconstriction showing that nettle acts directly on the vascular smooth muscle. The effect of AEN was most likely due to activation of α1-adrenergic receptors since prazosin blocked the response of the aorta to both N-Adr and AEN. Moreover, the contractile effects of AEN and N-Adr were not additive. It is surprising, though, that despite the strong α1-adrenergic effect of AEN in aorta, α1-adrenergic receptors do not seem to be involved in the cardiac effects of AEN. This is most likely because the negative chronotropic effect of AEN prevails over any putative additional effect due to activation of α1-adrenergic pathways.

In conclusion, we show here that nettle provokes an important bradycardia which is independent of cholinergic and α1-adrenergic receptors. This effect might at least partly account for the fast hypotensive action of this plant described *in vivo*. Our results demonstrate that nettle exerts an endothelium-independent vasoconstriction which appears rather contradictory to its hypotensive property. However, it is possible that this effect is masked *in vivo* by the important AEN-induced bradycardia. Alternatively, the effect of AEN may differ in small resistive vessels and capillaries, which are main determinants of blood pressure, from large vessels like the thoracic aorta studied here. The cardiac and vascular effects of AEN clearly involve different active principles. Phytochemical separation of these agents is needed to further identify their mechanisms of action.

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