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Diabetes abolishes the gender difference in vasopressin-mediated potentiation of sympathetic vasoconstriction

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Abstract

Electrical field stimulation (4 Hz, 0.2 ms pulse duration, at a supramaximal voltage of 70 V, for 1 s) of isolated rat tail artery segments produced contraction which was lower in female than in male rats, and was reduced by streptozotocin-induced diabetes in both genders. This contraction was potentiated by vasopressin (10^{-12}–10^{-10} M) more in normoglycemic male than in normoglycemic female rats, and this effect of vasopressin was increased by the cyclooxigenase inhibitor meclofenamate (10^{-5} M) in female control rats, but not in diabetic female, or control and diabetic male rats, and it was not modified by the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M). Endothelin-1 (10^{-10}–3×10^{-9} M) also potentiated the contraction to electrical stimulation. This potentiation was similar in all experimental groups, and it was not modified by meclofenamate or L-NAME. These results suggest that the potentiating effect of vasopressin, but not that of endothelin-1, on the sympathetic vasoconstriction, is lower in females than in males, probably by an increased release of vasodilating prostanoids, and this release may be reduced by diabetes in females.

Keywords: Caudal artery; Electrical stimulation; Male; Female; Nitric oxide; Prostanoid

1. Introduction

Pathologic cardiovascular changes are one of the most serious complications of diabetes mellitus (Nathan, 1993). Although cardiovascular diseases in the general, non-diabetic population are more frequent in men than in premenopausal women (Douglas, 1997; Hayward et al., 2000), diabetes may produce a relatively greater impairment in the female cardiovascular system, so the difference between men and premenopausal women regarding cardiovascular disease disappears in the diabetic patients (Farmer and Gotto, 1997; Haffner et al., 1997). Although there are some studies on the effects of exogenous sex hormones during diabetes (Bolego et al., 1999; Lim et al., 1999; Cignarella et al., 2000), studies comparing the effects of diabetes on the vascular reactivity in males and females are few. It has been found that diabetes reduces the vasodilatation to a nitric oxide donor in iliac arteries of male but not of female rats (Martinez-Nieves and Dunbar, 1999), that diabetes increases the contraction to prostanoids in the pulmonary circulation of male but not of female rats (Russ and Tobin, 1998), and diabetes abrogates the differences between women and men regarding nitric oxide-dependent relaxation (Steinberg et al., 2000).

One of the mechanisms that may underlie both diabetic vascular alterations and gender differences in cardiovascular regulation could be the characteristics of vascular reactivity to vasopressin and endothelin-1. It has been reported that the plasma levels of vasopressin (Brooks et al., 1989) and endothelin-1 (Takeda et al., 1991) may be elevated in diabetes. Studies about the effects of diabetes on the vascular response to these peptides report contradictory results. The effect of vasopressin on arterial blood pressure following ganglion blockade is reduced in diabetic rats (Hebden et al., 1987). Likewise, the vasoconstrictor effect of vasopressin is reduced in perfused kidneys (Sarubbi et al., 1989) and in the cutaneous circulation

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(Lawrence and Brain, 1992) of diabetic rats. On the contrary, the contraction to vasopressin is not modified by diabetes in basilar (Mayhan, 1998; Van Buren et al., 1998) and mesenteric arteries (Van Buren et al., 1998). Also, it has been reported that the vascular contraction to endothelin-1 may be increased (Tammesild et al., 1992; McIntyre et al., 2001), reduced (Lawrence and Brain, 1992; Guillon et al., 1998), or not modified (Mayhan, 1998) during diabetes. Moreover, the vascular effects of vasopressin and endothelin-1 may be different in male and female animals (Laycock and Whitehead, 1995). Both higher (Wang et al., 1997; Crofton et al., 1988) and lower (Altura, 1975; Stallone et al., 1991) vascular responses to vasopressin in males compared to females have been reported, whereas the response to endothelin-1 may be higher in human saphenous veins (Ergul et al., 1998) and porcine coronary arteries (Miller et al., 1996) from males compared with those from females, or similar in coronary arteries from male and female dogs (Lamping and Nuno, 1996). Preliminary experiments from our laboratory suggest that there are no differences between male or female, normoglycemic or diabetic rats, in the contraction of the tail artery to vasopressin, nor in the relaxation to acetylcholine in vasopressin-precontracted tail arteries (unpublished observations).

The physiological roles of vasopressin and endothelin-1 in cardiovascular regulation are controversial, because the concentrations of both peptides in plasma are low. However, these peptides, at concentrations lower than those which produce vasoconstriction directly, may potentiate the vascular response to sympathetic stimulation (García-Villalón et al., 2000). Therefore, the aim of the present study was to analyse the potentiating effect of vasopressin and endothelin-1 on the sympathetic response, whether this effect is modified by diabetes in the arteries of male and female animals, as well as the role of nitric oxide and prostanoids in this potentiation. This was performed using the rat tail artery, which has a dense sympathetic innervation (Cassis et al., 1985). Diabetes was induced by injection of streptozotocin, a model of experimental diabetes frequently used (Tomlinson et al., 1992; Öztürk et al., 1996).

2. Methods

Thirteen male and 12 female Sprague–Dawley rats, weighting 200–350 mg at the beginning of the study, were used. This investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No 85-23, revised 1996) and the European Community guidelines for the use of experimental animals. In one group of male and female rats, diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg, dissolved in citrate buffer pH 4.5), and a second group of age-matched control rats received only the vehicle. All rats were housed in cages and allowed free access to food and water. The concentration of glucose in plasma was determined from a drop of blood from the tail using Glucostix reactive strips (Bayer Diagnostics). Glucose determination was performed before and two days after streptozotocin injection, and again on the day of the experiment. In the female rats, the stage of the estrous cycle in the day of the experiment was determined by microscopic examination of vaginal smears. As no differences were found in the vascular responses from animals in the different days of the cycle, the results from all female animals were pooled.

Six weeks after streptozotocin or vehicle injection, the rats were killed by pentobarbitone overdose (200 mg/kg) followed by exsanguination, and the ventral caudal (tail) artery was dissected free and cut into cylindrical segments 2 mm in length. Each segment was prepared for isometric tension recording in a 4-ml organ bath at 37 °C, containing modified Krebs-Henseleit solution with the following composition (millimolar): NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Briefly, the method consists of passing through the lumen of the vascular segment of two fine stainless steel pins, 100 μm in diameter. One pin is fixed to the organ bath wall, while the other is connected to a strain gauge for isometric tension recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments), a Statham Microscale Accessory UL5 (Statham Instruments) and a Beckman Type RS Recorder (model R-411, Beckman Instruments). A previously determined optimal resting passive tension of 0.75 g was applied to the vascular segments, and then they were allowed to equilibrate for 60–90 min.

Electrical field stimulation (4 Hz, 0.2 ms pulse duration, at supramaximal voltage of 70 V, for 1 s) was applied to the arteries via two platinum electrodes placed on either side of the artery, and connected to a CS-14 stimulator (Cibertec). An interval of 10 min was imposed between stimulation periods to allow recovery of the response, and the stimulation was first repeated under control conditions until the responses were reproducible during over 40 min. Previous studies (García-Villalón et al., 2000; García-Villalón et al., 1999) indicate that the stimulation frequency used (4 Hz) produces a submaximal contraction which is optimal to show the potentiating effects of vasopressin and endothelin-1, and that the contraction to this stimulation is mainly due to stimulation of sympathetic perivascular nerve endings (García-Villalón et al., 2000).

After the repeated stimulations under control conditions were recorded, the effects of arginine-vasopressin (10^{-12}–10^{-10} M) and endothelin-1 (10^{-10}–3×10^{-9} M) on the

arterial response to electrical field stimulation were tested. Each of these substances was added cumulatively to the organ bath, and one electrical stimulus was applied 10 min after adding each concentration of the substance. In each experiment, a vascular segment which received electrical stimulation but was not treated with any substance was used as a time control.

The effect of vasopressin and endothelin-1 on the response to electrical stimulation was studied in the arteries in the absence and in the presence of the cyclooxygenase inhibitor meclofenamate (10^{-5} M) or the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M). After obtaining a reproducible, control response to electrical stimulation, one of these blockers was added to the organ bath, and after 20 min of incubation one electrical stimulus was applied; then vasopressin or endothelin-1 was also added and electrical stimulation was again applied in the presence of both the blocker and vasopressin or endothelin-1. To avoid desensitization, only one curve with vasopressin or endothelin-1 was performed in each vascular segment, and the effects of these peptides in the presence of meclofenamate or L-NAME were compared with the effects in a control segment from the same animal.

The values of the contraction to electrical stimulation are shown in absolute values, and the effects of vasopressin or endothelin-1 on the response to electrical stimulation as absolute increments over the control response. These data are expressed as means ± S.E.M. To compare the contraction to electrical stimulation and the increments in this contraction produced by vasopressin or endothelin-1, in arteries from male and female, control and diabetic animals, a two-way analysis of variance (ANOVA) was applied to these data, followed by unpaired Student's *t* test with the Bonferroni correction to determine which differences were significant. To compare the increments in the contraction produced by vasopressin or endothelin-1 in the absence or in the presence of meclofenamate or L-NAME, a one-way ANOVA was applied to these data, followed by Dunnet's *t* test to compare each condition with its control. $P < 0.05$ was considered significant.

Drugs used were: N^{ω}-nitro-L-arginine methyl ester (L-NAME); [Arg^8]-vasopressin acetate, and meclofenamate (2[1,6-Dichloro-3-methylphenyl-amino]benzoic acid, sodium salt); from Sigma, and endothelin-1 (human, porcine) from Peninsula Laboratories. All drugs were dissolved in distilled water and further diluted in isotonic NaCl.

3. Results

Six weeks after treatment with streptozotocin, male and female rats showed higher glycemia values ($P < 0.001$) and lower body weight ($P < 0.01$) than age-matched control rats (Table 1). Body weight was higher in male than in female, control and diabetic rats ($P < 0.001$), but glycemia values in control rats, or in streptozotocin-treated rats, were similar in the corresponding male and female animals (Table 1).

Table 1
Weight and glycemia values, and contraction of tail artery to electrical field stimulation (EFS, 4 Hz), from male and female rats, in control conditions and 6 weeks after diabetes induced by streptozotocin injection

	Males		Females	
	control	diabetic	control	diabetic
Weight (g)	367 ± 12	204 ± 26[d]	256 ± 7[b]	188 ± 6[c]
Glycemia (mg/dl)	96 ± 5	430 ± 12[d]	85 ± 3	394 ± 6[d]
Contraction to EFS (g)	0.77 ± 0.03	0.52 ± 0.05[d]	0.66 ± 0.022[a]	0.47 ± 0.03[c]
Number of animals	7	6	7	5

Values are means ± S.E.M. [a,b]Significantly different from control males ([a]$P < 0.05$; [b]$P < 0.001$). [c,d]Significantly different from normoglycemic controls ([c]$P < 0.01$; [d]$P < 0.001$).

Electrical stimulation (4 Hz) produced contraction of rat tail arteries, which was higher in arteries from male than in those from female rats ($P < 0.05$), and this contraction was reduced in arteries from diabetic animals, both male ($P < 0.001$) and female ($P < 0.01$) (Table 1). In time control experiments, only small variations (less than 5% increase or reduction in the response) were observed throughout the duration of the experiment (60 min).

Treatment with meclofenamate produced a slight reduction (0.02–0.1 g) of the contraction to electrical stimulation, and this reduction was not significantly different on arteries from males and females, control or diabetic rats. Treatment with L-NAME increased the contraction to electrical stimulation, and this increase was not significantly different in arteries from control female (0.19 ± 0.035 g) or from control male rats (0.34 ± 0.062 g). Also, the increase produced by L-NAME was not modified by diabetes in males (0.21 ± 0.053 g) or females (0.16 ± 0.035 g) compared to normoglycemic controls.

Vasopressin, at the concentrations used (10^{-12}–10^{-10} M), did not produce contraction by itself, but it increased significantly the contraction to electrical field stimulation in a concentration-dependent way (Fig. 1). In some cases, during the lowest concentrations of vasopressin (10^{-12} M–3×10^{-12} M), a small reduction in the response to electrical stimulation was observed, but this reduction was not significantly different from the variation in the time control segments. The increase produced by vasopressin was higher in arteries from normoglycemic male than in those from normoglycemic female rats (for vasopressin 10^{-11} M, the increases were 0.1 ± 0.02 vs. 0.006 ± 0.01 g; $P < 0.01$), and it was not modified significantly in arteries from diabetic male or female rats (for vasopressin 10^{-11} M, the increases were, respectively, 0.08 ± 0.01 and 0.07 ± 0.03 g) compared with their normoglycemic

controls. The increases produced by vasopressin were not significantly different in arteries from diabetic males compared with diabetic females. Treatment with meclofenamate increased ($P < 0.05$), the potentiating effect of vasopressin in the arteries of control female rats (for vasopressin 10^{-11} M, the increase in the presence of meclofenamate was 0.07 ± 0.02 g) but not in the arteries of diabetic female, or control or diabetic male rats. L-NAME did not modify significantly the potentiation by vasopressin in the arteries of control or diabetic, male or female rats.

Endothelin-1 (10^{-10}–3×10^{-9} M) by itself did not contract the tail arteries, but it increased the contraction to electrical field stimulation in a concentration-dependent way (Fig. 2) (for endothelin-1 10^{-9} M, the increases were 0.07 ± 0.02 g for normoglycemic males and 0.07 ± 0.016 g for normoglycemic females). As it was observed with vasopressin, in some cases, during the lowest concentration of endothelin-1 (10^{-10} M) a small reduction in the response to electrical stimulation was observed, but it was not significantly different from the variation in the time control segments. The potentiating effect of endothelin-1 was not significantly different in the arteries from male or female, control or diabetic rats, nor was it modified by treatment with meclofenamate or L-NAME.

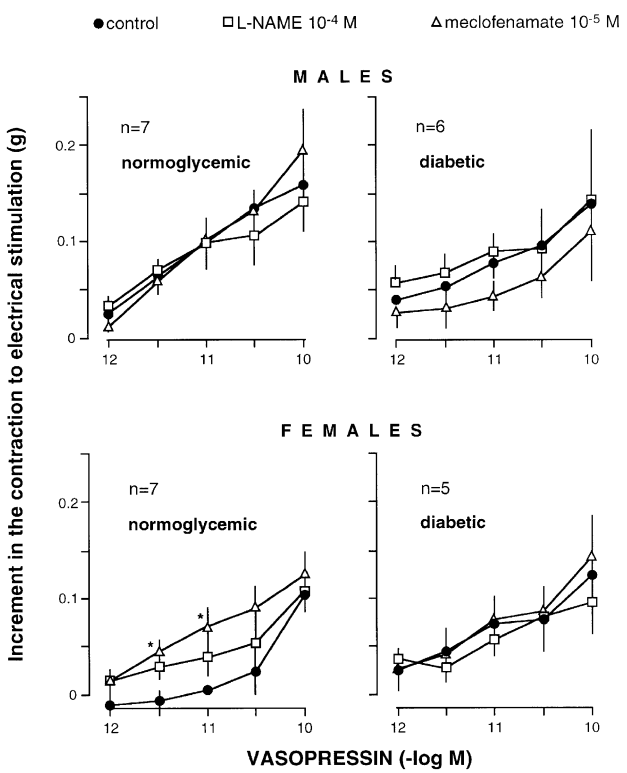

Fig. 1. Effect of vasopressin (10^{-12}–10^{-10} M) on the contraction to electrical stimulation (4 Hz) in tail arteries from normoglycemic (left panels) or from diabetic (right panels), male (upper panels) or female (lower panels) rats, in the absence (control) and in the presence of meclofenamate (10^{-5} M) or L-NAME (10^{-4} M). Values are means ± S.E.M. * Statistically significant ($P < 0.05$) compared to control ($P < 0.05$). n = number of animals.

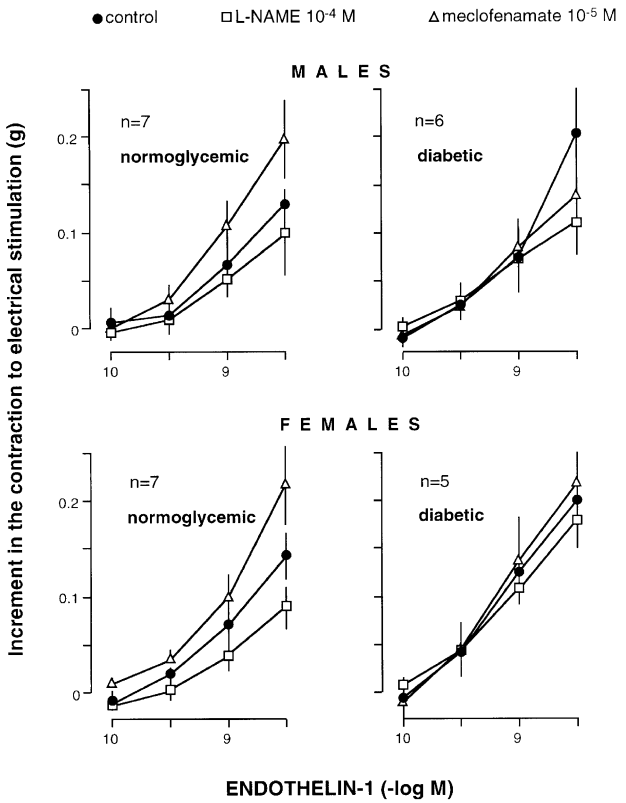

Fig. 2. Effect of endothelin-1 (10^{-10}–3×10^{-9} M) on the contraction to electrical stimulation (4 Hz) in tail arteries from normoglycemic (left panels) or from diabetic (right panels), male (upper panels) or female (lower panels) rats, in the absence (control) and in the presence of meclofenamate (10^{-5} M) or L-NAME (10^{-4} M). Values are means ± S.E.M. n = number of animals.

4. Discussion

Our results suggest that certain aspects of vascular reactivity may differ between males and females, and may be affected by diabetes differently in both genders.

First, we have found that the arterial contraction to electrical stimulation was lower in the arteries from female than in those from male rats in normoglycemic conditions (control). This agrees with studies performed by others (Li and Duckles, 1994). Similarly, lower responses to phenylephrine and potassium in arteries from females compared to those from males have been reported (Sánchez et al., 1996). This lower contraction may be due to the effects of estrogens on the vascular reactivity, and this might be related to the lower incidence of cardiovascular disease in premenopausal women compared with men (Douglas, 1997). Moreover, we have observed that the contraction to electrical stimulation was reduced in the arteries from

diabetic rats, both male and female, compared to normoglycemic controls. This phenomenon also has been observed before (Hart et al., 1988), and may be related to the damage of sympathetic nerve fibers and impaired sympathetic function by hyperglycemia (Monckton and Pehowich, 1980). In fact, autonomic dysfunction is a common complication in diabetic patients (Foster, 1998). There also may exist differences in the arterial diameter and/or amount of vascular smooth muscle between normoglycemic and diabetic animals, or between male and female rats, and these morphological differences may also contribute to the different contraction responses in these experimental groups. However, these morphological differences may not affect the maximum contractile response of the arteries, as the direct contraction produced by vasopressin in these arteries was similar in male and female, or in diabetic and normoglycemic rats (unpublished results), therefore, the differences observed in the contraction to electrical stimulation may be specific for this response.

Both vasopressin and endothelin-1 potentiated the arterial contraction to sympathetic stimulation, and this potentiation occurred at low peptide concentrations, which did not produce contraction by themselves. A previous study (García-Villalón et al., 2000) from our laboratory indicates that these peptide concentrations do not modify the contraction of these arteries to exogenous noradrenaline. Therefore, this potentiating effect may be specific for the interaction of endothelin-1 and vasopressin with the sympathetic response, and these peptides may participate in cardiovascular regulation by modulating the response to sympathetic vasoconstriction, in addition to the direct vasoconstricting effect that they may induce at higher concentrations. Interestingly, the concentrations of these peptides which produced this modulating effect may occur in vivo in some situations. The concentrations of vasopressin (10^{-12}–10^{-10} M) used in the present study are in the range which is found in plasma in physiological conditions (5×10^{-12} M; Gariepy et al., 1992). Although the concentrations of endothelin-1 used (10^{-10}–3×10^{-9} M) are higher than the plasma concentrations described for this peptide (10^{-12}–5×10^{-12} M; Frelin and Guedin, 1994), it has been found that endothelin-1 is released mainly in an abluminal direction (Wagner et al., 1992) and, therefore, it may reach local concentrations in the vascular wall higher than those in plasma. Therefore, these peptides may be of relevance for vascular regulation in physiological conditions.

The observed potentiating effect of endothelin-1 was similar in arteries from males and females, and it was also similar in the arteries from diabetic and normoglycemic rats. This suggest that this endothelin-1 effect is not involved in the gender differences in cardiovascular function, nor in the vascular alterations produced by diabetes. Some studies report reduced contraction to endothelin-1 in arteries from diabetic rats (Lawrence and Brain, 1992; Guillon et al., 1998) and this has been related to desensitization produced by elevated plasma levels of this peptide during diabetes (Clozel et al., 1993). Other studies, contrarily, have described increase (Tammesild et al., 1992; McIntyre et al., 2001), or no modification (Mayhan, 1998) in the endothelin-1-induced response during diabetes. These differences may be related to the intensity and/or duration of the diabetic state. Moreover, in the present study, the potentiating effect by endothelin-1 was not modified after inhibition of cyclooxygenase or nitric oxide synthase, although the concentrations of meclofenamate (10^{-5} M) (Szarek et al., 1998) and L-NAME (10^{-4} M) (Fernández et al., 1994) used may be sufficient to inhibit these enzymes. These results suggest therefore that this effect of endothelin-1 is independent of prostanoids and nitric oxide.

In the potentiating effects of vasopressin, we have found gender differences which may be related to the cardiovascular regulation in males and females, and to the vascular effects of diabetes in both genders. The potentiation produced by vasopressin on the sympathetic contraction was markedly higher in arteries from males compared to females. Previous studies have observed that gender differences in the response to vasopressin may vary when in vivo and in vitro results are compared. Increased in vitro contraction to this peptide has been found in aorta (Stallone et al., 1991) and mesenteric arteries (Altura, 1975; Stallone, 1995) from female compared to those from male rats. On the contrary, the increase of arterial pressure, and the increase of renal and mesenteric vascular resistance after vasopressin injection, were higher in males than in females (Wang et al., 1997). Our present results may explain this discrepancy between in vivo and in vitro results. The gender differences we have found in the potentiating effect of vasopressin may be specific for this interaction with the sympathetic response, as the direct contraction produced by vasopressin on the same vascular preparation was similar in males and females (unpublished results). As in vivo there is a basal sympathetic activity in most vascular beds, the in vivo effects of vasopressin may be mediated in part by the interaction of vasopressin with the sympathetic activity, and this may not occur for the in vitro response where this sympathetic activity is not present.

Our results also provide some information about the mechanisms of these gender differences in the effects of vasopressin. To analyze the role of nitric oxide and of prostanoids in these mechanisms, we used L-NAME and meclofenamate, which block the enzymes nitric oxide synthase and cyclooxygenase, respectively. The potentiation by vasopressin in the arteries from female control rats was increased by meclofenamate, suggesting that this effect of vasopressin may be reduced in these arteries by the production of vasodilating prostaglandins. However, in the arteries from control male rats this effect of meclofenamate was absent, suggesting that in males this modulating effect of prostaglandins may not exist. Therefore, the reduced potentiation by vasopressin observed in the arter-

ies from females may be related to a higher production of vasodilating prostaglandins in arteries from females compared to those from males. In agreement with this, it has been shown that estradiol increases, and testosterone reduces, prostacyclin release in cultured vascular smooth muscle cells (Wakasugi et al., 1989). The potentiating effect of vasopressin was not modified significantly by L-NAME, therefore, nitric oxide may not modulate this effect, as it was observed for endothelin-1 effect. We have observed that neither L-NAME nor meclofenamate modifies the direct contraction to higher concentrations of vasopressin in any of the experimental groups (unpublished results), therefore, the role of prostanoids in the arteries from female rats may be specific for the modulatory effect of low concentrations of vasopressin.

Regarding the effects of diabetes on the results with vasopressin, we have found that although the potentiation by this peptide was not significantly different in arteries from normoglycemic and diabetic rats, our results suggest that diabetes may alter the mechanisms of vasopressin action. In the arteries from female diabetic rats, the potentiating effect of vasopressin was not modified by meclofenamate, contrasting with that observed in arteries from normoglycemic female rats, in which meclofenamate increased the potentiating effect of vasopressin. This suggests that the probable modulator role of prostaglandins in the vasopressin potentiating effect, observed in control female rats, may be reduced in diabetic females. In relation to this, it has been described that the production of relaxing prostaglandins may be reduced by increased glucose concentrations (Ono et al., 1998). If the release of vasodilatory prostaglandins is reduced by diabetes, it should be expected that the potentiating effect of vasopressin would be increased in diabetic compared to normoglycemic females. Indeed, we have observed a marked tendency to an increase of the vasopressin effect in diabetic females, although this increase did not reach statistical significance. This may be due to a greater dispersion of the data from the diabetic animals which prevents the analysis from reaching the significance level. Also, diabetes may produce other effects on the vascular response to vasopressin (e.g. a reduction in the number of vasopressin receptors), which could partly offset the increase that should be expected due to the reduced release of prostanoids. However, this lack of significant increase during diabetes may not be incompatible with a reduction in vasodilator prostanoids during diabetes, as the effect of vasopressin in arteries from diabetic females was similar to that in arteries from normoglycemic females in the presence of meclofenamate.

The potentiating effect of vasopressin was similar in the arteries from diabetic male and in those from diabetic female rats, contrasting with the differences found between normoglycemic male and female rats. Also, in the arteries from diabetic or normoglycemic male rats, meclofenamate did not modify the potentiating effect of vasopressin, similarly to that observed in the arteries from diabetic females. Therefore, the effects of vasopressin and meclofenamate were different between normoglycemic male and female animals but similar between diabetic male and female rats, and thus, diabetes eliminated the difference between males and females in this particular aspect of cardiovascular reactivity. It may be hypothesized that in control conditions there is a higher release of vasodilator prostanoids in the arteries from females than in those from males, but that this difference is abolished by diabetes. This phenomenon may be of relevance to the physiopathology of diabetes in male and female patients. The incidence of cardiovascular disease is lower in premenopausal women compared to that in men, and this may be due in part to a greater production of vasodilating factors in the blood vessels of women (Hayward et al., 2000). However, diabetes produces an increase of cardiovascular alterations relatively greater in women than in men, so diabetes eliminates the "advantage" of women in this regard (Steinberg et al., 2000). This could be related to the reduction in the production of vasodilating factors, which could contribute to the apparition of cardiovascular complications as hypertension or coronary ischemia after diabetes.

In summary, our results suggest that both vasopressin and endothelin-1 at low subthreshold concentrations potentiate the sympathetic vasoconstriction, that this effect of vasopressin is reduced in arteries from females due to a greater production of vasodilating prostaglandins, and that this gender difference is eliminated by diabetes.

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References

Altura, B.M., 1975. Sex and estrogens responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J. Pharmacol. Exp. Ther. 193, 403–412.

Bolego, C., Cignarella, A., Zancan, V., Pinna, C., Zanardo, R., Puglisi, L., 1999. Diabetes abolishes the vascular protective effects of estrogen in female rats. Life Sci. 64, 741–749.

Brooks, D.P., Nuttin, D.F., Crofton, J.T., Share, L., 1989. Vasopressin in rats with genetic and streptozotocin-induced diabetes. Diabetes 38, 54–57.

Cassis, L.A., Stitzel, R.E., Head, R.J., 1985. Hypernoradrenergic innervation of the caudal artery of the spontaneously hypertensive rat: an influence upon neuroeffector mechanisms. J. Pharmacol. Exp. Ther. 234, 792–803.

Cignarella, A., Bolego, C., Pinna, C., Zanardo, R., Eberini, I., Puglisi, L.,

2000. The influence of sex hormones on vascular responses in the aorta of streptozotocin-diabetic male rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 361, 514–520.

Clozel, M., Loffler, B.M., Breu, V., Hilfiger, L., Maire, J.P., Butscha, B., 1993. Downregulation of endothelin receptors by autocrine production of endothelin-1. Am. J. Physiol. 265, C188–C192.

Crofton, J.T., Share, L., Brooks, D.P., 1988. Pressor responsiveness to and secretion of vasopressin during the estrous cycle. Am. J. Physiol. 255, R1041–R1048.

Douglas, P.S., 1997. Coronary artery disease in women. In: Braunwald, E. (Ed.), Heart Disease. A Textbook of Cardiovascular Medicine. W.B. Saunders, Philadelphia, pp. 1704–1714.

Ergul, A., Shoemaker, K., Puett, D., Tackett, R.L., 1998. Gender differences in the expression of endothelin receptors in human saphenous veins in vitro. J. Pharmacol. Exp. Ther. 285, 511–517.

Farmer, J.A., Gotto, A.M., 1997. Dyslipidemia and other risk factors for coronary artery disease. In: Braunwald, E. (Ed.), Heart Disease. A Textbook of Cardiovascular Medicine. W.B. Saunders, Philadelphia, pp. 1126–1157.

Fernández, N., Monge, L., García-Villalón, A.L., García, J.L., Gómez, B., Diéguez, G., 1994. Cooling effects on nitric oxide production by rabbit ear and femoral arteries during cholinergic stimulation. Br. J. Pharmacol. 113, 550–554.

Foster, D.W., 1998. Diabetes mellitus. In: Fauci, A.S., Braunwald, E., Isselbacher, K.J., Wilson, J., Martin, J.B., Kasper, D.L., Hauser, S.L., Longo, D.L. (Eds.), Harrison's Principles of Internal Medicine. McGraw-Hill, New York, pp. 2060–2081.

Frelin, C., Guedin, D., 1994. Why are circulating concentrations of endothelin-1 so low? Cardiovasc. Res. 28, 1613–1622.

García-Villalón, A.L., Monge, L., Fernández, N., Sánchez, M.A., Martínez, M.A., Gómez, B., Diéguez, G., 1999. Basal inhibitory action of endogenous endothelin on the sympathetic contraction in the isolated rat tail artery. Eur. J. Pharmacol. 384, 163–167.

García-Villalón, A.L., Monge, L., Fernández, N., Sánchez, M.A., Martínez, M.A., Gómez, B., Diéguez, G., 2000. Impaired potentiation by endothelin-1 and vasopressin of sympathetic contraction in tail artery from hypertensive rats. Cardiovasc. Res. 45, 463–469.

Gariepy, L., Larose, P., Baileiy, B., du Souich, P., 1992. Effect of lignocaine on arginine-vasopressin plasma levels: baseline or induced by furosemide. Br. J. Pharmacol. 106, 470–475.

Guillon, J.M., Thiry, C., Roach, A.G., Cavero, I., 1998. Preferential reduction in vascular responses to endothelin-1 in rats with streptozotocin-induced diabetes. J. Cardiovasc. Pharmacol. 31 (Suppl. 1), S133–S137.

Haffner, S.M., Miettinen, H., Stern, M.P., 1997. Relatively more atherogenic coronary heart disease risk factors in prediabetic women than in prediabetic men. Diabetologica 40, 711–717.

Hart, J.L., Freas, W., McKenzie, J.E., Muldoon, S.M., 1988. Adrenergic nerve function and contractile activity of the caudal artery of the streptozotocin diabetic rat. J. Auton. Nerv. Syst. 25, 49–57.

Hayward, C.S., Kelly, R.P., Collins, P., 2000. The roles of gender, the menopause and hormone replacement on cardiovascular function. Cardiovasc. Res. 46, 28–49.

Hebden, R.A., Bennett, T., Gardiner, S.M., 1987. Pressor sensitivities to vasopressin, angiotensin II, or methoxamine in diabetic rats. Am. J. Physiol. 253, R726–R734.

Lamping, K.G., Nuno, D.W., 1996. Effects of 17β-estradiol on coronary microvascular responses to endothelin-1. Am. J. Physiol. 271, H1117–H1124.

Lawrence, E., Brain, S.D., 1992. Altered microvascular reactivity to endothelin-1, endothelin-3 and N^G-nitro-L-arginine methyl ester in streptozotocin-induced diabetes mellitus. Br. J. Pharmacol. 106, 1035–1040.

Laycock, J.F., Whitehead, S.A., 1995. Vasopressin and vascular regulation: is sex a factor? J. Endocrinol. 144, 389–392.

Li, Z., Duckles, S.P., 1994. Influence of gender on vascular reactivity in the rat. J. Pharmacol. Exp. Ther. 268, 1426–1431.

Lim, S.C., Caballero, A.E., Arora, S., Smakowski, P., Bashoff, E.M., Brown, F.M., Logerfo, F.W., Horton, E.S., Veves, A., 1999. The effect of hormonal replacement therapy on the vascular reactivity and endothelial function of healthy individuals and individuals with type 2 diabetes. J. Clin. Endocrinol. Metab. 84, 4159–4164.

Martinez-Nieves, B., Dunbar, J.C., 1999. Vascular dilatory responses to sodium nitroprusside (SNP) and α-adrenergic antagonism in female and male normal and diabetic rats. Proc. Soc. Exp. Biol. Med. 222, 90–98.

Mayhan, W.G., 1998. Constrictor responses of the rat basilar artery during diabetes mellitus. Brain Res. 783, 326–331.

McIntyre, C.A., Hadoke, P.W., Williams, B.C., Lindsay, R.M., Elliott, A.I., McKnight, J.A., 2001. Selective enhancement of sensitivity to endothelin-1 despite normal endothelium-dependent relaxation in subcutaneous resistance arteries isolated from patients with type I diabetes. Clin. Sci. (Colch) 100, 311–318.

Miller, V.M., Barber, D.A., Fenton, A.M., Wang, X., Sieck, G.C., 1996. Gender differences in response to endothelin-1 in coronary arteries: transcription, receptors and calcium regulation. Clin. Exp. Pharmacol. Physiol. 23, 256–259.

Monckton, G., Pehowich, E., 1980. Autonomic neuropathy in the streptozotocin diabetic rat. Can. J. Neurol. Sci. 7, 135–142.

Nathan, D.M., 1993. Long-term complications of diabetes mellitus. N. Engl. J. Med. 328, 1676–1685.

Ono, H., Umeda, F., Inoguchi, T., Ibayashi, H., 1998. Glucose inhibits prostacyclin production by cultured aortic endothelial cells. Thromb. Haemostasis 60, 174–177.

Öztürk, Y., Altan, V.M., Yildizoglu-Ari, N., 1996. Effects of experimental diabetes and insulin on smooth muscle functions. Pharmacol. Rev. 48, 69–112.

Russ, R.D., Tobin, B.W., 1998. Differential pulmonary vascular effects of streptozotocin diabetes in male and female rats. Proc. Soc. Exp. Biol. Med. 217, 74–80.

Sánchez, A., Gómez, M.J., Dorantes, A.L., Rosales, J.L., Pastelin, G., Díaz, V., Posadas, F., Escalante, B., 1996. The effect of ovariectomy on depressed contractions to phenylephrine and KCl and increased relaxation to acetylcholine in isolated arotic rings of female compared to male rabbits. Br. J. Pharmacol. 118, 2017–2022.

Sarubbi, D., McGiff, J.C., Quilley, J., 1989. Renal vascular responses and eicosanoid release in diabetic rats. Am. J. Physiol. 257, F762–F768.

Stallone, J.N., 1995. Mesenteric vascular responses to vasopressin during development of DOCA-salt hypertension in male and female rats. Am. J. Physiol. 268, R40–R49.

Stallone, J.N., Crofton, J.T., Share, L., 1991. Sexual dimorphism in vasopressin-induced contraction of rat aorta. Am. J. Physiol. 260, H453–H458.

Steinberg, H.O., Paradisi, G., Cronin, J., Crowde, K., Hempfling, A., Hook, G., Baron, A.D., 2000. Type II diabetes abrogates sex differences in endothelial function in premenopausal women. Circulation 101, 2040–2046.

Szarek, J., Spurlock, B., Gruetter, C.A., Lemke, S., 1998. Substance P and capsaicin release prostaglandin E2 from rat intrapulmonary bronchi. Am. J. Physiol. 275, L1006–L1012.

Takeda, Y., Miyamori, I., Yoneda, T., Takeda, R., 1991. Production of endothelin-1 from the mesenteric arteries of streptozotocin induced diabetic rats. Life Sci. 48, 2553–2556.

Tammesild, P.J., Hodgson, W.C., King, R.G., 1992. Increased sensitivity to endothelin-1 in isolated Kreb's-perfused kidneys of streptozotocin diabetic rats. Clin. Exp. Pharmacol. Physiol. 19, 261–265.

Tomlinson, K.C., Gardiner, S.M., Hebden, R.A., Bennett, T., 1992. Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol. Rev. 44, 103–150.

Van Buren, T., Vleeming, W., Krutzen, M.M., Van de Kuil, T., Gispen, W.H., De Wildt, D.J., 1998. Vascular responses of isolated mesenteric resistance and basilar arteries from sort- and long-term diabetic rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 663–670.

Wagner, O.F., Christ, G., Woita, J., Vierhapper, H., Parzer, S., Nowotny, P.J., Scheneider, B., Waldhausi, W., Binder, B.R., 1992. Polar secretion of endothelin-1 by cultured endothelial cells. J. Biol. Chem. 267, 16066–16068.

Wakasugi, M., Noguchi, T., Kazama, Y., Kanemaru, Y., Onaya, T., 1989. The effects of sex hormones on the synthesis of prostacyclin (PGI2) by vascular tissues. Prostaglandins 37, 401–410.

Wang, Y.-X., Crofton, J.T., Bealer, S.L., Share, L., 1997. Sexual dimorphism in regional blood flow responses to vasopressin in conscious rats. Am. J. Physiol. 273, R1126–R1131.