Contractile responses of human thyroid arteries to vasopressin

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ABSTRACT

Aims: In the present study we investigated the intervention of nitric oxide and prostacyclin in the responses to vasopressin of isolated thyroid arteries obtained from multi-organ donors.
Main methods: Paired artery rings from glandular branches of the superior thyroid artery, one normal and the other deendothelised, were mounted in organ baths for isometric recording of tension. Concentration–response curves to vasopressin were determined in the absence and in the presence of either the vasopressin V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ (10^{-8} M), the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M), or the inhibitor of prostaglandins indomethacin (10^{-6} M).
Key findings: In artery rings under resting tension, vasopressin produced concentration-dependent, endothelium-independent contractions. The vasopressin V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ (10^{-8} M) displaced the control curve to vasopressin 19-fold to the right in a parallel manner. The contractile response to vasopressin was unaffected by L-NMMA or by indomethacin.
Significance: Vasopressin causes constriction of human thyroid arteries by stimulation of V_1 vasopressin receptors located on smooth muscle cells. These effects are not linked to the presence of an intact endothelium or to the release of nitric oxide or prostaglandins. The constriction of thyroid arteries may be particularly relevant in certain pathophysiological circumstances in which vasopressin is released in amounts that could interfere with the blood supply to the thyroid gland.

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Introduction

Vasopressin is a neuropeptide that promotes reabsorption of water in renal tubular cells through vasopressin-V_2 receptors and produces contraction of vascular smooth muscle through vasopressin-V_1 receptors (Michell et al., 1979; Penit et al., 1983; Barrett et al., 2007). With regard to human vessels, vasopressin causes powerful V_1 receptor-mediated constriction in isolated mesenteric (Ohlstein and Berkowitz, 1986; Martínez et al., 1994b), cerebral (Lluch and Gómez, 1987; White and Robertson, 1987) and renal (Medina et al., 1996b) arteries. This effect is endothelium-independent and due to direct stimulation of receptors located on smooth muscle cells (Martín de Aguilera et al., 1990; Martínez et al., 1994a, b). Both hyperthyroidism and hypothyroidism are associated with marked effects on the cardiovascular system including changes in cardiac output, blood pressure, and systemic vascular resistance (Klein and Ojamaa, 2001). On the other

hand, impaired thyroid function is accompanied by changes in plasma vasopressin levels (Arnaout et al., 1992; Marcisz et al., 2001) that might be responsible for constriction of thyroid vessels. The effects of vasopressin in these arteries are not fully known. Such data might be of pathophysiological significance in those states characterised by increased plasma vasopressin levels, such as haemorrhagic and septic shock (Landry et al., 1997; Tsuneyoshi et al., 2003) and in some patients with congestive heart failure (Rouleau et al., 1994; Goldsmith and Gheorghiade, 2005). Studies from our laboratory have shown that in human thyroid arteries from multiorgan donors the endothelium modulates responses to acetylcholine and noradrenaline through the release of nitric oxide (NO), an endothelium-derived relaxing factor (Torondel et al., 2004; Ortega et al., 2005). Moreover, NO production is decreased in hyperthyroid patients when compared with control subjects (Hermenegildo et al., 2002). These results strongly suggest that endothelial NO might play a key role in the responsiveness of thyroid arteries to specific pharmacological interventions. Accordingly, the aim of the present study was to determine the effects of vasopressin in human thyroid arteries obtained from multiorgan donors. Observations were made in the presence and in the absence of endothelium and after exposure to N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, or the vasopressin V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$.

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Material and methods

Subjects

Glandular branches of the superior thyroid artery were obtained from 12 multiorgan donors during procurement of organs for transplantation (8 men and 4 women; age range: 22–65 years; cause of death: 7 head trauma, 2 intracranial haemorrhage, 3 myocardial infarction). The study was approved by the ethical committee of our institution. The vessels were immediately placed in a Petri dish containing refrigerated (4 °C) modified Krebs–Henseleit solution of the following mM composition: NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgCl_2·6H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose 11.1 and disodium EDTA, 0.01.

In vitro experiments

Experiments started within 2 h, or not more than 8 h after organs were removed. When used within 2 to 8 h, the vessels were stored in the 4 °C modified Krebs-Henseleit solution, which was found not to alter either the endothelium-dependent relaxation to acetylcholine or the contractile response to KCl or vasopressin. Arteries were cleaned of connective tissue and cut into rings (3 mm in length, 1 to 1.5 mm outer diameter) under a dissecting microscope. In some experiments, the endothelium was removed mechanically by inserting a roughened stainless-steel wire into the lumen and gently rolling the vessel ring on wet filter paper. Each ring was suspended between two stainless-steel L-shaped pins in 4-mL organ baths containing modified Krebs-Henseleit solution. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH from 7.3 to 7.4 and maintained at 37 °C with a circulating water jacket and a heat pump. One pin was fixed to the organ bath wall and the other was connected to a strain gauge (model FT03; Grass Instrument Division of Astro-Med Inc., West Warwick, RI). Changes in isometric force were recorded on a Macintosh computer (Apple Computer, Cupertino, CA, USA) by use of Chart version 3.4/s software and a MacLab/8e data acquisition system (ADInstruments, Mountain View, CA, USA). To establish the resting tension for maximal force development, we performed a series of preliminary experiments on thyroid artery rings which were exposed repeatedly to 100 mM KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 10 mN. The rings were allowed to attain a steady level of tension during a 2- to 3-hour accommodation period before testing. The contractile response to 100 mM KCl was similar in intact and denuded arteries (24.7 ± 4.8 versus 23.9 ± 4.1 mN; $n = 8$, P > 0.05).

Functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-7} to 10^{-6} M) during contraction obtained with noradrenaline (10^{-6} to 3×10^{-6} M). Arteries in which the acetylcholine reversed the noradrenaline-induced tone by more than 70% were designated as endothelium intact and arteries in which acetylcholine caused less than 15% relaxation were designated as denuded.

Concentration–response curves to vasopressin were determined in a cumulative manner under the following conditions: (1) in the absence of inhibitors (control response); (2) in the presence of the V_1-receptor antagonist 1-(β-mercapto-β,β-cyclopentamethylene-propionic acid) 2-(O-methyl)-tyrosine, 8-arginine) vasopressin (d(CH_2)$_5$Tyr(Me)AVP, 10^{-8} M); (3) in the presence of L-NMMA (10^{-4} M) to inhibit NO synthase; and (4) in the presence of indomethacin (10^{-6} M) to inhibit the production of PGI_2. Control (in the absence of inhibitors) and experimental (after incubation for 20 min with inhibitors) responses were obtained from separate artery rings.

Chemicals

All substances were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Data analysis

All values are expressed as means $\pm$ SEM. Contractile effects were expressed as a percentage of the response to KCl (100 mM). EC_{50} values (concentration of agonist producing half-maximum effect) were expressed as pD_2 ($-\log EC_{50}$). The maximum contractions and pD_2 values were compared by one-way analysis of variance (ANOVA) with the Bonferroni test as post hoc test. n values are presented as the number of donors. Statistical significance was accepted at P < 0.05.

Results

KCl (100 mM) produced a sustained contraction with a maximal effect of 24.3 ± 3.1 mN (Fig. 1). In precontracted arteries with noradrenaline (10^{-6} M), acetylcholine (10^{-6} M) caused relaxation in arteries with an intact endothelium, but was abolished in endothelium-denuded rings (Fig. 1). Cumulative application of vasopressin produced a constrictor response that was concentration-dependent (Fig. 1). The maximum tension developed as well as the pD_2 were similar (P > 0.05) in arterial segments with and without endothelium (Fig. 1 and Table 1). The presence of the vasopressin V_1-selective receptor antagonist d(CH_2)$_5$Tyr(Me) AVP (10^{-8} M) in the organ bath shifted the concentration–response curve to vasopressin 19-fold (P < 0.05) to the right in a parallel manner, but differences in the maximum tensions developed were not significant (P > 0.05) (Fig. 1D and Table 1).

The presence of L-NMMA (10^{-4} M) did not change significantly (P > 0.05) the concentration–response curve for vasopressin (Fig. 2 and Table 1). However, L-NMMA decreased acetylcholine-induced relaxation in artery rings precontracted with noradrenaline (10^{-6}–3×10^{-6} M), thus showing the ability of these vessels to display NO-mediated relaxation (Fig. 2B). Arteries exposed to indomethacin (10^{-6} M) did not show significant changes in the contractile response to vasopressin (Fig. 2 and Table 1).

Discussion

Our results indicate that vasopressin is a potent agonist for the contraction of thyroid vascular smooth muscle through activation of specific V_1-vasopressin receptors. The maximum tensions with respect to KCl response and sensitivity (in terms of pD_2 values) attained with vasopressin in thyroid arteries are within the range of values obtained in other human arteries (Medina et al., 1997; Aldasoro et al., 2008) but higher than the corresponding values in human saphenous vein (Aldasoro et al., 1997; Medina et al., 1997).

The functional properties of the endothelium–NO system of human thyroid arteries remain largely unexplored. NO synthase, the enzyme responsible for the formation of NO (Palmer et al., 1988) is present in the thyroid follicular cells and in endothelial cells of the human thyroid gland (Colin et al., 1997), and human thyrocites produce the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) (Colin et al., 1995; Millatt et al., 2000). In addition, NO modulates the response of human thyroid arteries to acetylcholine and noradrenaline (Torondel et al., 2004). Previously, we have demonstrated in human thyroid arteries that treatment with L-NMMA reduces the endothelium-dependent relaxation to acetylcholine. The NO component, sensitive to L-NMMA, accounted for approximately 20% of the maximum relaxation to acetylcholine. The NO-independent relaxation results from the release by acetylcholine of endothelium-dependent hyperpolarizing factor (EDHF). The EDHF component involves activation of Ca^{2+}-dependent K^+ channels, sensitive to charybdotoxin and apamin (Torondel et al., 2004).

All these results are consistent with a role for NO in responsiveness of thyroid arteries. However, the intervention of NO seems to be restricted to specific pharmacological receptors. Indeed the present experiments show that the vasopressin-induced contraction is not linked to the presence of an intact endothelium. The evidence for this
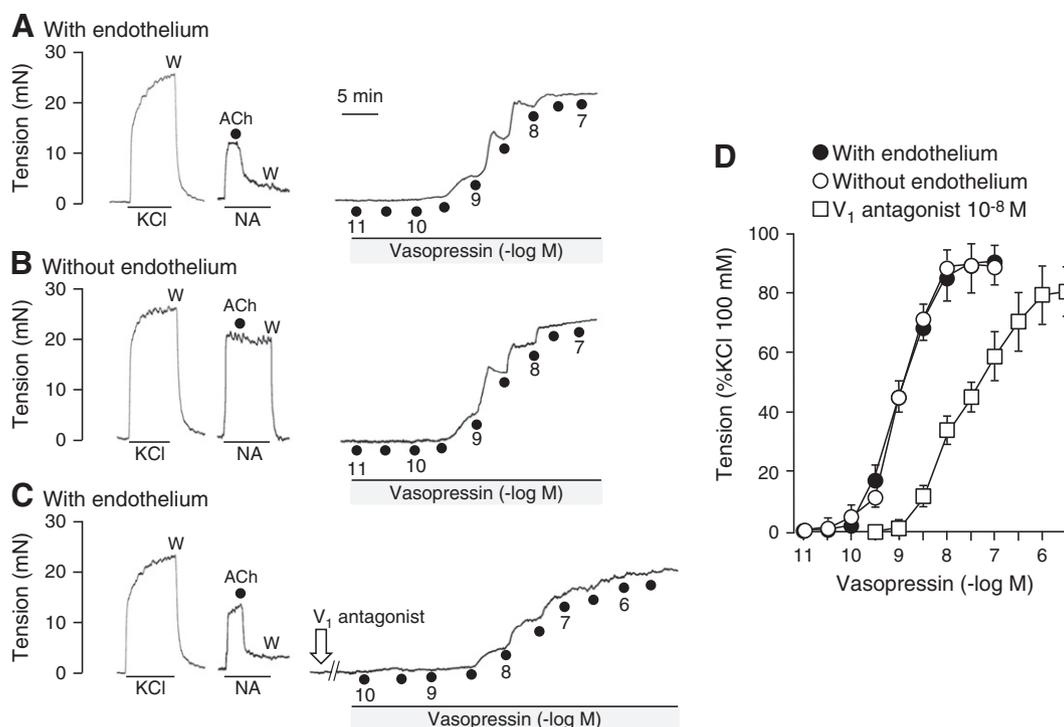
Fig. 1. Effects of vasopressin in human thyroid artery. Recordings illustrating the effects of KCl (100 mM), noradrenaline (NA, 10^{-6} M), acetylcholine (ACh, 10^{-6} M) and vasopressin (10^{-11}–10^{-6} M) in thyroid artery rings A) with and B) without endothelium, and C) in the presence of 10^{-8} M of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP. Points mark time of addition of drugs and (W) marks washout. D) Concentration–response curves for vasopressin in arterial rings with ($\bullet$, $n = 7$) and without ($\circ$, $n = 6$) endothelium, and in the presence of 10^{-8} M of the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP ($\square$, $n = 6$); results are expressed as a percentage of the contraction developed by 100 mM KCl. Values are means ± SEM.

is that the contraction was similar in intact and endothelium-denuded arteries. In agreement with the present results, previous experiments have shown that vasopressin causes endothelium-independent contraction in several human arteries (Martínez et al., 1994a, b; Medina et al., 1996b; Segarra et al., 1998). Furthermore, the contraction does not involve the intervention of the L-arginine-NO pathway because L-NMMA, a selective inhibitor of NO synthase, did not modify the vasopressin-induced contraction. The present results are consistent with previous data showing that NO synthase inhibition does not affect the vasopressin-induced contractions in human arteries (Medina et al., 1996a,b). However, in vitro and in vivo experiments indicate that a considerable regional heterogeneity exists in the reactivity of blood vessels to vasopressin. In isolated rabbit saphenous, coronary and renal arteries, and to a minor degree in basilar arteries, the endothelium has an inhibitory effect on the vasoconstriction induced by vasopressin mediated by the release of NO (García-Villalón et al., 1996).

As vasopressin has been reported to induce vasodilating prostaglandin synthesis via a V_1 receptor-dependent pathway (Nakatani et al.,

Table 1
pD$_2$ values and maximum contractions (Emax) to vasopressin in the different experimental conditions in segments of human thyroid arteries.

Artery rings	pD$_2$	Emax (%)
With endothelium ($n = 7$)	8.97 ± 0.25	88 ± 6
Without endothelium ($n = 6$)	8.93 ± 0.24	90 ± 5
L-NMMA (10^{-4} M) ($n = 5$)	9.00 ± 0.19	95 ± 7
Indomethacin (10^{-6} M) ($n = 5$)	8.87 ± 0.21	78 ± 5
d(CH$_2$)$_5$Tyr(Me)AVP (10^{-8} M) ($n = 6$)	7.69 ± 0.33[*]	77 ± 7

n = number of donors; pD$_2$ = negative logarithm of molar concentration required to produce half-maximum response. Emax is expressed as a percentage of responses to 100 mM KCl. Values are mean ± SEM.
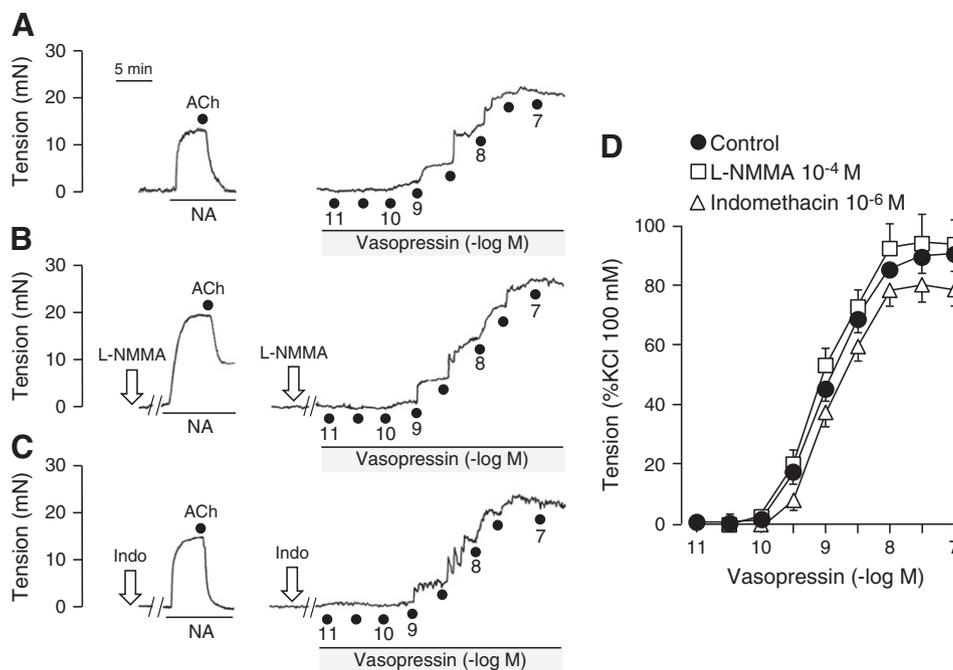[*] P < 0.05 vs artery segments with endothelium.

2007) it might be expected that cyclooxygenase inhibitors can modulate the responses to vasopressin. Indomethacin potentiates the vasopressin-induced contraction in human mesenteric (Martínez et al., 1994b) and renal (Medina et al., 1996b) arteries with or without endothelium, thus suggesting that relaxant prostaglandins, derived mainly from smooth muscle cells, attenuate the V_1-mediated contractile effect of vasopressin. However, contractile responses of human thyroid artery to vasopressin were unaffected by cyclooxygenase inhibition, thus suggesting that in this vessel, vasopressin does not stimulate the release of prostanoids. A common finding in the vascular effects of vasopressin is the heterogeneity of responsiveness, depending on regional and species differences (García-Villalón et al., 1996). Therefore, it is likely that the contractile effects of vasopressin in thyroid arteries are due to direct stimulation of specific receptors located on smooth muscle cells.

d(CH$_2$)$_5$Tyr(Me)AVP has been reported to be a potent inhibitor of the contractile response to vasopressin in human arteries (Martínez et al., 1994a,b; Medina et al., 1996b; Segarra et al., 1998). Furthermore, this compound does not interfere with the vasoconstrictor response to angiotensin II, noradrenaline or KCl (Ohlstein and Berkowitz, 1986; Fox et al., 1987). The present results show that d(CH$_2$)$_5$Tyr(Me)AVP is an effective antagonist of the response to vasopressin in human thyroid arteries. At 10^{-8} M, the antagonist produced a 19-fold shift to the right of the control concentration–response curve to vasopressin, presumably due to a competitive agonist–antagonist interaction.

Plasma levels of vasopressin increase in response to stimuli such as increased plasma osmolality (Mason, 1980), dehydration, hypotension (Sorenson and Hammer, 1985), exercise (Melin et al., 1980), increased angiotensin II levels (Bonjour and Malvin, 1970), and sympathetic activation (Leng et al., 1999) and in different pathophysiological settings such as, haemorrhage (Fujisawa et al., 1994), and congestive heart failure (Preibisz et al., 1983). In addition, vasopressin may modify the effects of other vasoactive substances that are found in plasma or released from perivascular nerve endings. Several studies have reported

Fig. 2. Recordings illustrating the effects of noradrenaline (NA, 10^{-6} M), acetylcholine (ACh, 10^{-6} M) and vasopressin (10^{-11}–10^{-7} M) in thyroid artery rings with endothelium A) in the absence and B) in the presence of L-NMMA (10^{-4} M) or C) indomethacin (10^{-6} M). D) Responses to vasopressin in the absence (●, $n = 7$) and in the presence of 10^{-4} M of L-NMMA (□, $n = 5$) or 10^{-6} M of indomethacin (△, $n = 5$); results are expressed as a percentage of the contraction developed by 100 mM KCl. Values are means ± SEM.

a significant augmentation by vasopressin of the vasoconstricting action of catecholamines (Medina et al., 1997; Segarra et al., 1998). Consequently, the vascular effects of vasopressin on thyroid artery may be particularly relevant in those states characterised by increased vasopressin plasma levels as well as in those clinical situations, such as asystolic cardiac arrest, refractory arterial hypotension during anaesthesia, septic shock, or oesophageal variceal haemorrhage, in which vasopressin and synthetic vasopressin receptor agonists are used as a therapeutic tool (Treschan and Peters, 2006). Under these conditions the contractile response of the thyroid artery induced by vasopressin may reduce thyroid blood flow and thyroid hormone release. Interestingly, along with increased levels of vasopressin, low thyroid hormone circulating levels have been described in patients with heart failure (Hamilton et al., 1990) and are associated with poor prognosis (Iervasi et al., 2003). These findings suggest a potential link between the vasoconstrictive effects of vasopressin on thyroid arteries and low plasmatic thyroid hormone concentrations associated with unfavourable outcomes of these patients. Nevertheless, we cannot deduce from our observations whether disease-related increases in plasma vasopressin would reduce thyroid blood flow enough to significantly affect thyroid function. Future studies will therefore be needed to define the role of vasopressin in the regulation of thyroid blood flow.

In view of the specificity and potency of the vasopressin V_1-receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP, it seems appropriate to consider the use of vasopressin receptor antagonists as a potentially beneficial therapeutic approach in circumstances in which vasopressin plasma concentrations are markedly raised (e.g. congestive heart failure). Indeed, several vasopressin antagonists, orally active in humans, are presently undergoing clinical development (Arai et al., 2007).

Conclusions

Our study shows that vasopressin exerts a powerful constrictor action on isolated human thyroid arteries by direct stimulation of V_1-vasopressin receptors located on smooth muscle cells. It appears that in these arteries the contractile response to vasopressin is not modulated by the NO and cyclooxygenase pathways.

Conflict of interest statement

The authors declared no conflict of interest.

Acknowledgements

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