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Neurotensin induces the release of prostacyclin from human umbilical vein endothelial cells in vitro and increases plasma prostacyclin levels in the rat

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Abstract

Human umbilical vein endothelial cells express high affinity neurotensin receptors which are coupled to phosphoinositide turnover and $^{45}Ca^{2+}$ efflux (Schaeffer et al., 1995. J. Biol. Chem. 270, 3409-3413). In order to assess the physiological significance of neurotensin receptor activation in endothelial cells, we have compared the in vitro effect of neurotensin on prostacyclin release and cytosolic free calcium increase ($[Ca^{2+}]_i$) as determined by fura-2 fluorescence experiments to the in vivo effect of neurotensin on blood pressure and haematocrit. Neurotensin increased $[Ca^{2+}]_i$ levels at low concentrations ($EC_{50} = 4.2 \pm 0.2$ nM, $n = 3$). At similar concentrations, neurotensin was also able to induce prostacyclin release from human umbilical vein endothelial cells ($EC_{50} = 14 \pm 1$ nM, $n = 3$) as determined by a 6-keto-prostaglandin $F_{1\alpha}$ enzyme immunoassay. The neurotensin (100 nM)-induced $[Ca^{2+}]_i$ increase and prostacyclin release were inhibited by the specific non-peptide neurotensin receptor antagonist SR 48692 at similar concentrations ($IC_{50} = 41 \pm 16$ nM and 86 ± 17 nM, respectively, $n = 3$), confirming that these responses were mediated by high affinity neurotensin receptors. Intravenous injection of neurotensin (1–4 nmol/kg i.v.) in the rat resulted in a drop of blood pressure and increased haematocrit, and nearly doubled the plasma levels of 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin. Whereas indomethacin (10 mg/kg i.v.) pretreatment significantly reduced the effect of neurotensin on blood pressure, it did not alter its effect on haematocrit. These results suggest that prostacyclin release plays a role in the hypotensive effects of neurotensin, but is not involved in its effects on haematocrit.

Keywords: Neurotensin; Prostacyclin; Umbilical vein endothelial cell, human; Hematocrit

1. Introduction

Intravenous (i.v.) injection of the tridecapeptide neurotensin elicits a host of different cardiovascular effects (for review see Kitabgi et al., 1985). Actually, neurotensin may induce hypotensive, hypertensive or both effects in sequence depending on the species and the experimental conditions (Carraway and Leeman, 1973; Quirion et al., 1980; Bachelard et al., 1992). Furthermore, i.v. injection of neurotensin results in increased haematocrit as a consequence of vascular permeability increases and associated oedema (Carraway et al., 1991) but neurotensin analogues have also been shown to inhibit heat-induced rat paw oedema (Gao and Wei, 1995). This complexity of effects of neurotensin has precluded the elucidation of the precise sequence of events leading to the cardiovascular effects of the peptide. However, several mechanisms have so far been described as being implicated in the cardiovascular effects of neurotensin: (i) release of histamine from mast cells and subsequent vasodilatation resulting in hypotension (Quirion et al., 1980), (ii) activation of capsaicin-sensitive nerve terminals resulting in hypertensive effects (Bachelard et al., 1992) and (iii) catecholamine release from the adrenal medulla (Bachelard et al., 1992).

None of the mechanisms described up to now thus involves direct effects of neurotensin on the cells of the vascular wall, only indirect effects having been reported. However, we have recently shown that high affinity neurotensin receptors exist on human umbilical vein endothelial cells and that they are functionally coupled to intracellular Ca^{2+} release. Neurotensin binding sites were also

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detected on human aortic endothelial cells, suggesting that neurotensin may directly influence vasoreactivity through endothelial cell activation (Schaeffer et al., 1995). Endothelial cells play a major role in cardiovascular regulation, essentially through the release of modulators like nitric oxyde (NO), prostacyclin and endothelins (Lüscher and Vanhoutte, 1990). Furthermore, the barrier function of endothelial cells is a major determinant of the vessel wall permeability and loss of this function results in blood protein leakage and oedema (Lum and Malik, 1994). Endothelial neurotensin receptors would therefore be ideally situated to participate in most of the cardiovascular actions of neurotensin. The two major vasodilatory mediators synthesised in endothelial cells are NO and prostacyclin. We have shown previously that neurotensin is not able to increase intracellular cGMP levels in human umbilical vein endothelial cells, whereas sodium nitroprusside is a potent activator, showing that guanylate cyclase is present in these cells, but cannot be activated by neurotensin (Schaeffer et al., 1995). In order to clarify the potential physiological consequences of endothelial neurotensin receptor activation, we therefore focussed on prostacyclin, the second vasodilatory substance besides NO likely to be released after endothelial cell activation. These in vitro effects were compared with the in vivo effects of neurotensin on prostacyclin plasma concentrations.

2. Materials and methods

2.1. Materials

RPMI 1640 medium and phosphate-buffered saline were from Biochrom (Poly Labo, Strasbourg, France). Fetal calf serum and human fibronectin were from Boehringer Mannheim (Meylan, France). Heparin and endothelial cell growth supplement were from Sigma (Saint-Quentin Fallavier, France). Neurotensin was from Bachem (Voisins-Le-Bretonneux, France). Human umbilical vein endothelial cells were obtained from Clonetics (TEBU, Paris, France). 6-Keto-[^{3}H]prostaglandin $F_{1\alpha}$ was from Amersham (Les Ulis, France). Fura-2 acetoxymethyl ester (fura-2/AM) was from Molecular Probes (Eugene, OR, USA).

2.2. Cell culture

Human umbilical vein endothelial cells were routinely cultured in 75 cm^2 flasks coated with human fibronectin (5 $\mu g/cm^2$) in RPMI 1640 medium containing 10% fetal calf serum, antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml), heparin (100 μg/ml) and endothelial cell growth supplement (30 μg/ml). For experiments, trypsin/EDTA-detached cells were seeded in fibronectin-coated 24-well plates and used at confluence. Human umbilical vein endothelial cells were used from the third to the seventh passage.

2.3. Cytosolic free calcium ($[Ca^{2+}]_i$) measurements

Human umbilical vein endothelial cells cultured in 75 cm^2 flasks were detached with non-enzymatic cell dissociation solution (Sigma, France), scraped from the flasks, centrifuged and resuspended in physiological salt solution (PSS, composition: NaCl 145 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1 mM, glucose 5.6 mM, HEPES/NaOH 5 mM pH 7.4), containing fura-2/AM (1 μM) and incubated at 37°C for 30 min. The cell suspension was then diluted five times with PSS, and incubated for a further 60 min at 37°C. After two washes with PSS to remove extracellular fura-2, cells were resuspended in PSS and kept in the dark at room temperature. Experiments were carried out under constant stirring in a PTI spectrofluorometer using approximately 75 000 cells in 3 ml fluorescence cuvettes at 37°C. $[Ca^{2+}]_i$ was calculated from the fluorescence ratio $R = \frac{F_{340}}{F_{380}}$ (where F_{340} and F_{380} are the fluorescence intensities of fura-2 measured at 510 nm after excitation at 340 nm and 380 nm, respectively) as described by Grynkiewicz et al. (1985).

2.4. Prostacyclin release measurements

Human umbilical vein endothelial cells (maximum passage 4) were grown to confluence on fibronectin (5 $\mu g/cm^2$)-coated 24-well plates. Experiments were carried out as described by Royston et al. (1992), using prostacyclin release values during a 2-h preincubation period to correct for basal release. Prostacyclin released during 1 h of stimulation with neurotensin was measured as its stable degradation product 6-keto-prostaglandin $F_{1\alpha}$ using an enzyme immunoassay kit from Amersham (Les Ulis, France). Antagonists were preincubated for 5 min before the addition of neurotensin. Experiments were carried out in triplicate.

2.5. Determination of plasma 6-keto-prostaglandin $F_{1\alpha}$ levels, haematocrit and mean arterial blood pressure in the rat

Male Sprague-Dawley rats weighing about 300 g (3 months) were obtained from Iffa Crédo (L'Arbesle, France). The animals had free access to standard diet and to tap water. They were subdivided at random in groups of 5 animals. In order to measure 6-keto-prostaglandin $F_{1\alpha}$ levels and haematocrit, neurotensin extemporaneously dissolved in saline was injected i.v. at various doses 15 min before blood sample collection. Blood was collected into EDTA/indomethacin (EDTA 20 mg ml^{-1}/indomethacin 2 mM, 9:1) by aortic puncture of pentobarbital-anaesthetized rats (30 mg/kg i.p.). Samples were stored on ice, centrifuged and plasma was rapidly frozen and stored at −20°C. 6-Keto-prostaglandin $F_{1\alpha}$ was solid phase-extracted from blood samples following the instruc-

tions of the enzyme immunoassay kit from Amersham (Les Ulis, France) used for the determination of 6-keto-prostaglandin $F_{1\alpha}$ levels. Recovery was checked by spiking blood samples with trace amounts of 6-keto-[^{3}H]prostaglandin $F_{1\alpha}$ and was found to be around 80%. Haematocrit was measured with a Baker Instruments Haematology Cell Counter (series 8000, Rungis, France). To measure blood pressure, a catheter implanted into the right carotid artery of pentobarbital-anaesthetized rats (30 mg/kg i.p.) was connected to a Statham P 10 EZ pressure transducer coupled to a Gould RS 3400 recorder (Oxnard, CA, USA).

The in vivo protocols were approved by the Animal Care and Use Committee of Sanofi Recherche.

2.6. Data analysis

IC_{50}, EC_{50} values, slope factors and their standard errors concerning $[Ca^{2+}]_i$ data were determined by fitting the logistic equation to the data from individual experiments as described by DeLean et al. (1978). Identical procedures were used for prostacyclin release data with the exception that data from all the experiments were pooled and analysis carried out on the mean values because of the higher inherent variability of these experiments. All data are given as mean ± S.E.M. Plasma 6-keto-prostaglandin $F_{1\alpha}$ levels, as well as data concerning blood pressure and haematocrit, were compared by analysis of variance (ANOVA) followed by Duncan's multiple range test. The level of statistical significance was chosen as $P < 0.05$.

3. Results

3.1. Effect of neurotensin on $[Ca^{2+}]_i$ in human umbilical vein endothelial cells

As shown in Fig. 1A, neurotensin induced a transient increase of $[Ca^{2+}]_i$ in fura-2-loaded human umbilical vein endothelial cell suspensions. The time course of this $[Ca^{2+}]_i$ increase was very similar to the time course of $^{45}Ca^{2+}$ efflux induced by neurotensin as reported previously (Schaeffer et al., 1995), a peak response being reached after around 30 s. As shown in Fig. 1B, the effect of neurotensin on $[Ca^{2+}]_i$ in human umbilical vein endothelial cells was dose-dependent ($EC_{50} = 4.2 \pm 0.2$ nM, $n = 3$). The concentrations of neurotensin active on $[Ca^{2+}]_i$ were very similar to those activating $^{45}Ca^{2+}$ efflux in these cells ($EC_{50} = 4$ nM, Schaeffer et al., 1995). SR 48692 is the first specific non-peptide antagonist with high affinity to neurotensin receptors (Gully et al., 1993) and as such can be used as a selective tool to characterize neurotensin receptors. SR 48692 inhibited the neurotensin (100 nM)-induced $[Ca^{2+}]_i$ increase with an IC_{50} value of 41 ± 16 nM ($n = 3$, Fig. 1B inset), confirming that this $[Ca^{2+}]_i$ increase is mediated by high affinity neurotensin receptors.

3.2. Effect of neurotensin on prostacyclin release from human umbilical vein endothelial cells

Prostacyclin is unstable and rapidly degraded to the stable metabolite 6-keto-prostaglandin $F_{1\alpha}$, the level of which can be determined by enzyme-immunoassay tech-

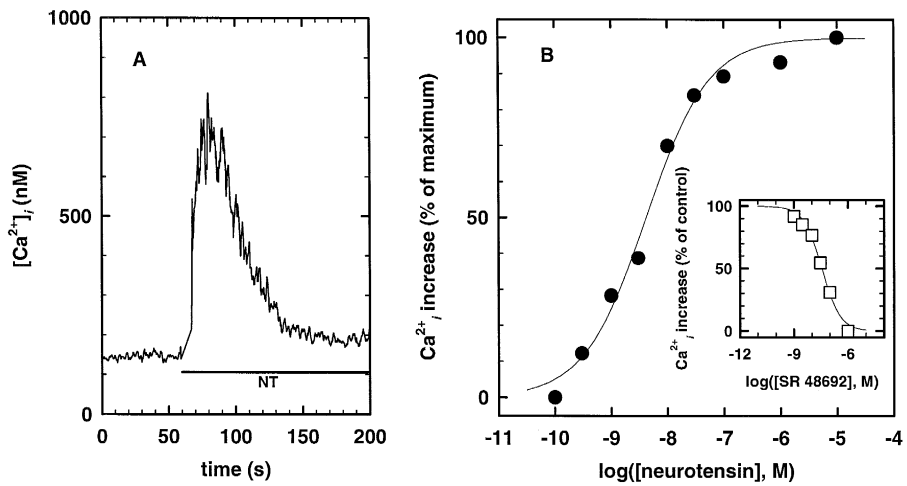

Fig. 1. Effect of neurotensin on $[Ca^{2+}]_i$ in human umbilical vein endothelial cells. (A) Representative tracing of the effect of neurotensin on human umbilical vein endothelial cells. Cells were stimulated with 10 μM of neurotensin (solid bar). $[Ca^{2+}]_i$ was determined from the fluorescence ratio $R = F_{340}/F_{380}$ as described in Section 2. (B) Human umbilical vein endothelial cells were stimulated with the indicated concentrations of neurotensin and peak Ca^{2+} concentrations determined as increases in fura-2 fluorescence ratio $R = F_{340}/F_{380}$. Results are expressed as a percentage of the maximal increase observed with 10 μM of neurotensin (480 ± 130 nM, $n = 3$) and are the mean of three determinations. Inset: Effect of SR 48692 on neurotensin (100 nM)-induced $[Ca^{2+}]_i$ increase in human umbilical vein endothelial cells. The antagonist was preincubated for 3 min before the addition of neurotensin. Results are expressed as a percentage of the control response to neurotensin (100 nM) and are the mean of 2–3 determinations. S.E.M. represented around 5% of the mean.

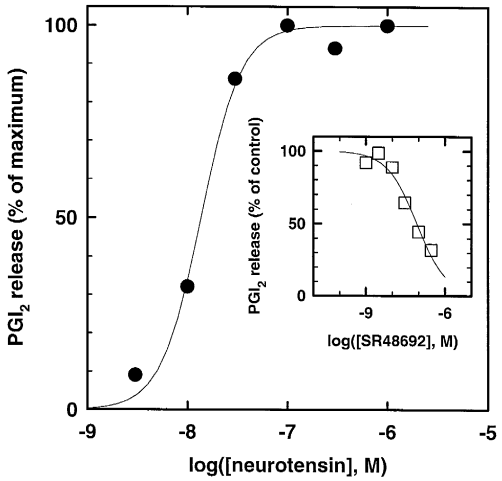

Fig. 2. Effect of neurotensin on prostacyclin release from human umbilical vein endothelial cell monolayers. Prostacyclin release during a 1 h incubation period was determined as its stable degradation product 6-keto-prostaglandin $F_{1\alpha}$ by enzyme-immunoassay. Results are expressed as a percentage of the maximal increase of 6-keto-prostaglandin $F_{1\alpha}$ induced by neurotensin (100 nM) and are the mean of 2–3 experiments performed in triplicate. Inset: Effect of SR 48692 on neurotensin (100 nM)-induced prostacyclin release from human umbilical vein endothelial cells. SR 48692 was preincubated for 5 min before the addition of neurotensin. Results are expressed as a percentage of the release induced by 100 nM of neurotensin in each experiment. Data are the mean of 2–4 experiments performed in triplicate. S.E.M. represented around 15% of the mean.

niques (Salmon, 1978). As shown in Fig. 2, neurotensin was able to increase prostacyclin release from human umbilical vein endothelial cells; the maximal release was 18 ± 9 pg/well per 60 min ($n = 3$). In the same experiments, thrombin (12 nM, maximal effect) increased prostacyclin release by 23 ± 9 pg/well per 60 min, showing that neurotensin was almost as effective as thrombin (not shown). The half-maximal effect of neurotensin was observed at concentrations ($EC_{50} = 14 \pm 1$ nM, $n = 3$) very similar to those inducing an increase in $[Ca^{2+}]_i$ in these cells (see above).

SR 48692 inhibited neurotensin (100 nM)-induced prostacyclin release in the same concentration range as the neurotensin-induced $[Ca^{2+}]_i$ increase ($IC_{50} = 86 \pm 16$ nM, $n = 4$). The similarity of the concentrations of neurotensin stimulating prostacyclin release and $[Ca^{2+}]_i$ increase as well as the inhibition by SR 48692 clearly indicate that high affinity neurotensin receptors are involved in the neurotensin-induced prostacyclin release. Furthermore, the effect of neurotensin on prostacyclin production was totally blocked by low concentrations of indomethacin ($IC_{50} = 180 \pm 30$ nM, $n = 3$, not shown), indicating that this effect of neurotensin can be blocked by inhibition of cyclooxygenase.

3.3. Effect of neurotensin on plasma 6-keto-prostaglandin $F_{1\alpha}$ levels in the rat

In order to assess the effect of neurotensin on prostacyclin production in vivo, circulating 6-keto-prostaglandin $F_{1\alpha}$ levels were measured after i.v. neurotensin administration in the rat. As shown in Fig. 3A, circulating 6-keto-prostaglandin $F_{1\alpha}$ levels 15 min after the administration were significantly and dose-dependently increased by neurotensin, a maximum effect being observed at doses higher than 2 nmol/kg. This neurotensin (2 nmol/kg)-induced prostacyclin elevation was blocked by indomethacin (10 mg/kg i.v., 15 min before neurotensin) and by SR 48692 (10 mg/kg p.o., 1 h before neurotensin) (Fig. 3B), showing that this increase was due to neurotensin receptor activation and subsequent stimulation of arachidonic acid metabolism through the cyclooxygenase pathway and was not due to non-specific effects. Mepyramine, a histamine H_1 receptor antagonist, was not able to significantly inhibit neurotensin-induced circulating 6-keto-prostaglandin $F_{1\alpha}$ levels, although a slight decrease was apparent (Fig. 3B).

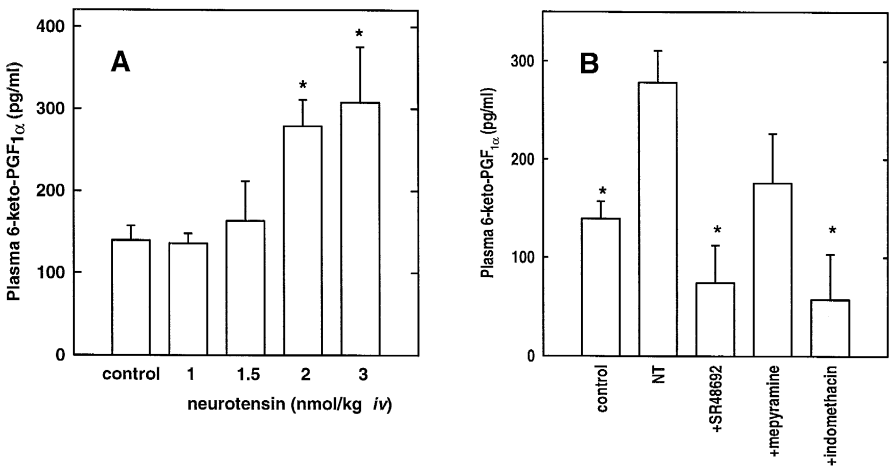

Fig. 3. Effect of i.v. neurotensin on the plasma levels of 6-keto-prostaglandin $F_{1\alpha}$ in the rat. Plasma levels of 6-keto-prostaglandin $F_{1\alpha}$ were determined 15 min after the injection of increasing doses of neurotensin (A) or neurotensin (NT, 2 nmol/kg) and antagonists (B): SR 48692 (10 mg/kg p.o., 1 h before the injection), mepyramine and indomethacin (10 mg/kg i.v., 15 min before the injection). Values are means ± S.E.M. ($n = 5$). Data were compared by ANOVA as described in Section 2 (* $P < 0.05$, A: significance vs. control; B: significance vs. NT).

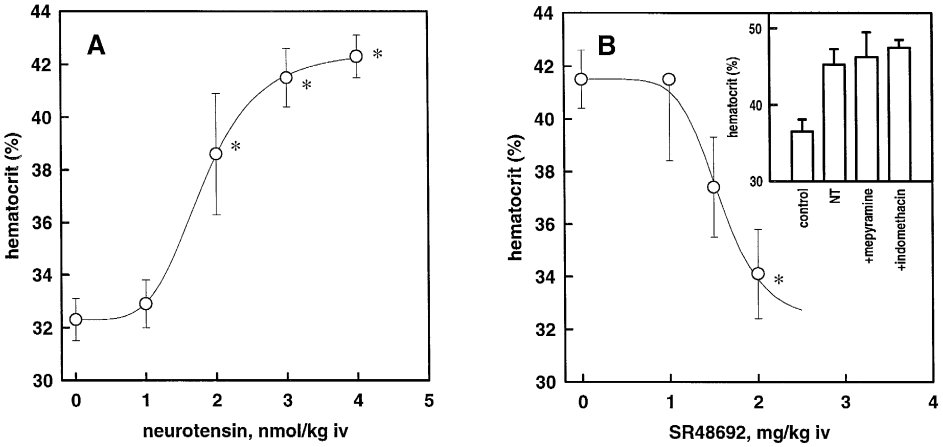

Fig. 4. Effect of neurotensin on the haematocrit in the rat. Haematocrit was determined 15 min after the i.v. injection of increasing doses of neurotensin (A) or neurotensin (2 nmol/kg) and SR 48692 (B) injected i.v. 5 min before neurotensin. Inset: Effect of mepyramine and indomethacin (10 mg/kg i.v., 15 min before neurotensin) on neurotensin (NT, 2 nmol/kg)-induced changes in haematocrit. Values are means ± S.E.M. ($n = 5$). Data were compared by ANOVA as described in Section 2 (* $P < 0.05$).

3.4. Effect of neurotensin on haematocrit and blood pressure

In order to determine whether any in vivo effects of neurotensin could be attributed to prostacyclin release, the effects of indomethacin on neurotensin-induced changes of haematocrit and blood pressure were also assessed. As shown in Fig. 4A, i.v. injection of increasing doses of neurotensin resulted in a dose-dependent increase in haematocrit. This effect could be blocked by low concentrations of SR 48692 (Fig. 4B), showing that this effect of neurotensin was due to neurotensin receptor activation. However, indomethacin and mepyramine even at high concentrations (10 mg/kg i.v., 15 min before neurotensin) did not affect this response of neurotensin (Fig. 4B inset).

Neurotensin injection has also been shown to induce strong hypotensive effects in the rat (Carraway and Leeman, 1973). As has been shown previously (Gully et al., 1996), this effect of neurotensin could be decreased by SR 48692 (10 mg/kg p.o., 1 h before neurotensin), and was partially but significantly antagonised by indomethacin and mepyramine (10 mg/kg i.v., 15 min before neurotensin) (Fig. 5).

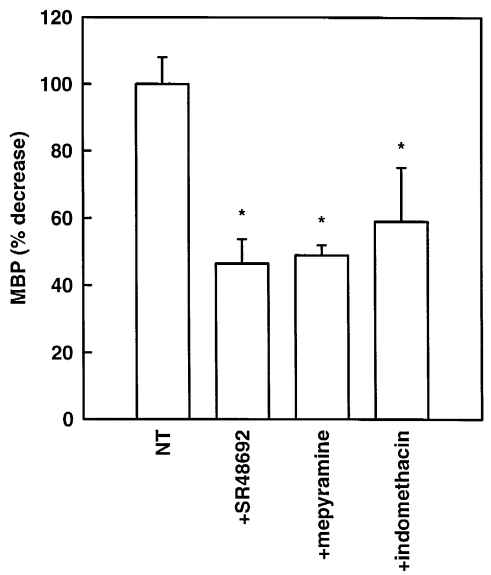

Fig. 5. Effect of neurotensin on rat mean arterial blood pressure. Mepyramine (10 mg/kg) and indomethacin (10 mg/kg) were injected i.v. 15 min before neurotensin (NT, 1 nmol/kg i.v.). SR 48692 was given p.o. 1 h before neurotensin. Mean arterial blood pressure (MBP) was determined 1 min after neurotensin injection and results expressed as a percentage of the neurotensin-induced depressor effect (corresponding to -50 ± 4 mmHg). Values are means ± S.E.M. ($n = 5$). Data were compared by ANOVA as described in Section 2 (* $P < 0.05$).

4. Discussion

In a previous paper, we have shown that human umbilical vein endothelial cells express high affinity neurotensin receptors (Schaeffer et al., 1995). Receptor activation leads to increased phosphoinositide metabolism and $^{45}Ca^{2+}$ efflux, which are classically associated with endothelial cell activation. In this paper we studied prostacyclin release as a mechanism through which endothelial cell neurotensin receptors may participate in the cardiovascular effects of neurotensin.

The neurotensin-induced prostacyclin release in human umbilical vein endothelial cells was prominent, because it reached almost 80% of the maximal effect of thrombin, which is one of the most potent prostacyclin releasers. Furthermore, prostacyclin release was observed at exactly the same concentrations of neurotensin as those which increase $[Ca^{2+}]_i$, and was blocked by the non-peptide neurotensin receptor antagonist SR 48692 at low concentrations, showing that it is due to high affinity neurotensin receptor activation. SR 48692 is very specific for neurotensin receptors and has been shown not to interact with a whole range of other receptors even at high concentrations (Gully et al., 1993). These in vitro data suggest

therefore that neurotensin-induced prostacyclin release may play a role in the cardiovascular effects of neurotensin. However, the implication of endothelium-derived vasodilatory mediators in the hypotensive effects of neurotensin has not been studied extensively. An endothelium-dependent relaxing effect of neurotensin has been reported in a dog carotid artery preparation (D'Orléans-Juste et al., 1985), whereas no relaxation after neurotensin was observed in the rat aorta (Di Paola and Richelson, 1990) and the rabbit pulmonary artery (Obara et al., 1989). Also, indomethacin inhibited the contractile effect of neurotensin in the rat portal vein (Rioux et al., 1980), but it has been reported to be ineffective on the hypotensive effect of neurotensin in the rat (Quirion et al., 1980). However, the fact that prostacyclin may have contractile properties in the venous vasculature (Warner, 1990) may complicate the outcome of in vivo experiments. According to all these reports, it is therefore not clear whether or not prostacyclin may play a role in the cardiovascular effects of neurotensin.

Clearly, however, two prerequisites would be necessary to attribute any effect of neurotensin to prostacyclin release: first, that neurotensin should be able to increase the circulating levels of prostacyclin to levels high enough to explain its effects, and secondly, that inhibition of prostacyclin production by indomethacin should inhibit the relevant in vivo effects of neurotensin. In order to determine whether the in vitro observations could be substantiated in the in vivo situation, plasma prostacyclin metabolite levels were measured. Actually, neurotensin injection approximately doubled the circulating 6-keto-prostaglandin $F_{1\alpha}$ levels, which are the levels attained by prostacyclin infusions giving dramatic pharmacological effects (Hensby et al., 1979). Furthermore, the absolute increase in circulating prostacyclin metabolite (more than 100 pg/ml) was similar to the levels of the prostacyclin analogue iloprost inducing significant cardiovascular effects (Grant and Goa, 1992). Moreover, the fact that mepyramine did not significantly inhibit this effect of neurotensin shows that the increase is not a consequence of neurotensin-induced histamine release (Quirion et al., 1980; Carraway et al., 1982; and see below). The increase in circulating prostacyclin metabolite levels therefore clearly appears consistent with the hypothesis that some of the effects of neurotensin may be prostacyclin mediated. The in vitro data would clearly favour the endothelium as the source of circulating prostacyclin, although smooth muscle cells have also been shown to be able to release prostacyclin (Daret et al., 1993; Williams et al., 1994). However, human aortic smooth cells do not express a significant number of neurotensin receptors (our own unpublished data).

The second condition for the implication of prostacyclin in a reponse to neurotensin, i.e., inhibition of the response by indomethacin, is only met by the hypotensive response to neurotensin. Actually, up to now the hypotensive effects of neurotensin have classically been attributed to neurotensin-induced histamine from mast cells, leading to secondary hypotension (Quirion et al., 1980; Carraway et al., 1982; Miller et al., 1995). However, it has also always been clear that the hypotensive effects of neurotensin depend on the experimental conditions, the response being highly sensitive to the presence or absence of anaesthetics and even depending on the particular anaesthetic used (Chahl and Walker, 1981; Bachelard et al., 1992). In unanaesthetized rats for instance, the hypotensive effects seemed to be masked by a pressure response to catecholamines released after neurotensin injection (Bachelard et al., 1992). This sensitivity to experimental conditions may explain why the implication of prostacyclin in the hypotensive effects of neurotensin has not been reported up to now (Quirion et al., 1980). Despite these discrepancies, the partial inhibition of the hypotensive effect of neurotensin by both indomethacin and mepyramine seen in the present work is in favour of adding one more level of complexity to the multifactorial mechanism of neurotensin-induced hypotension, with both mast cell-derived histamine, serotonin (Quirion et al., 1980) and endothelium-derived prostacyclin playing a role. Furthermore, it should be noted that the local haemodynamic effects of neurotensin in a specific vascular bed may be dependent to a distinct degree on these two mechanisms, as well as on pressor effects through catecholamines and capsaicin-sensitive nerves (Bachelard et al., 1992), the mean arterial pressure reflecting the combined effects of all of these mechanisms in different vascular beds.

By contrast, the absence of any inhibitory effect of indomethacin on neurotensin-induced haematocrit increase shows that prostacyclin does not play a major role in neurotensin-induced haemoconcentration. This is not entirely surprising because eicosanoids other than prostacyclin (i.e., leukotrienes) have been implicated in the elevation in haematocrit observed after neurotensin injection (Carraway et al., 1991).

In conclusion, this paper shows that activation of neurotensin receptors on human umbilical vein endothelial cells induces an increase in $[Ca^{2+}]_i$ and in prostacyclin production. Neurotensin injection results in circulating prostacyclin levels high enough to be consistent with a role of prostacyclin relase in the systemic effects of the peptide. The reduction of the hypotensive effects of neurotensin injection by indomethacin pretreatment strongly supports the hypothesis that part of the hypotensive effects of neurotensin injection can be attributed to prostacyclin release. By contrast, this is not the case for neurotensin-induced haemoconcentration which is not affected by indomethacin pretreatment.

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