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Bimodal effects of chronically administered neurokinin B (NKB) on *in vivo* and *in vitro* cardiovascular responses in female rats

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Abstract

The *in vivo* cardiovascular effects of acutely administered neurokinin B (NKB) have been attributed both to direct effects on vascular tone and to indirect effects on central neuroendocrine control of the circulation. We proposed: 1) that a modest long-term increase in plasma NKB levels would decrease mean arterial pressure (MAP) due to attenuated peripheral vascular tone, and 2) that chronic high-dose NKB would increase MAP, due to increased sympathetic outflow which would override the peripheral vasodilation. We examined the *in vivo* and *in vitro* cardiovascular effects of chronic peripheral NKB. Low- (1.8 nmol/h) or high- (20 nmol/h) dose NKB was infused into conscious female rats bearing telemetric pressure transducers. MAP, heart rate (HR) and the pressor responses to I.V. phenylephrine (PE, 8 μg) and angiotensin II (Ang II, 150 ng) were measured. Concentration–response curves of small mesenteric arteries were constructed to PE using wire myography. Low-dose NKB reduced basal MAP (88±2 mm Hg to 83±2 mm Hg), did not affect resting HR, reduced the pressor responses to PE, and attenuated the maximal constriction of mesenteric arteries to PE and KCl. By contrast, high-dose NKB increased basal MAP (86±1 mm Hg to 89±1 mm Hg), increased HR (350±3 beats/min to 371±3 beats/min), increased the pressor responses to Ang II and, contrary to our hypothesis, increased the maximum contractile responses of mesenteric arteries to PE and KCl. The cardiovascular effects of NKB are thus dose-dependent: whereas chronic low-dose NKB directly modulates vascular tone to reduce blood pressure, chronic high-dose NKB induces an increase in blood pressure through both central (indirect) and peripheral (direct) pathways.

Keywords: Neuromedin K; Pregnancy; Pre-eclampsia; Blood pressure; Vascular reactivity

1. Introduction

Neurokinin B belongs to a family of neuropeptides called tachykinins; these include substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and hemokinin-1 (HK-1) [22,25,35]. Although NKB was long believed to be exclusively of neuronal origin and activity, it is now recognized that it can act at G-protein-coupled receptors to induce changes in vascular tone, both directly on the smooth muscle cells, and indirectly through release of vasoactive factors from the endothelial cells [35]. The ultimate effect, constriction or dilation, depends on the relative tissue distribution of, and binding to, the NK receptor subtypes NK_1, NK_2 and NK_3. Although NK_3 is the preferred receptor for NKB, NKB does also bind to NK_1 and NK_2 receptors to induce physiological effects [35].

In addition to its presence in the central nervous system, NKB mRNA and immunoreactive peptide have now been identified in placental tissue [26], and the moderate increases in NKB expression in maternal and cord blood during pregnancy has been attributed to placental synthesis [6,7,26]. It has been suggested that NKB, levels of which are exceptionally high in pre-eclamptic women, may be responsible in part for the hypertension, intense vasoconstriction and proteinuria associated with the disorder [26,31]. Compared with normal pregnancy, pre-eclamptic women also exhibit increased sensitivity to pressor agents, especially to angiotensin II (Ang II) [2,13,14]. The mechanism(s) by which NKB might contribute to such changes in cardiovascular regulation are not known.

The *in vivo* effects of acutely administered NKB on cardiovascular regulation have not been consistent. NKB is generally regarded as a depressor agent, although it may cause an

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increase or decrease in systemic blood pressure depending on the route of administration, state of consciousness, species and dose [35]. There is evidence that NKB may, in addition to its direct peripheral vascular effects, act centrally to raise systemic blood pressure through increased vasopressin release and sympathetic outflow [39]. *In vitro*, NKB causes endothelium-dependent vasodilation in arteries through activation of NK_1 receptors [5]. However, NKB-induced vasoconstriction has also been observed in pulmonary and coronary arteries through activation of NK_2 receptors [15,30,35]. The cardiovascular effects of acutely adistered NKB are thus complex. It could directly alter total peripheral resistance, and/or it could act indirectly to influence central control of blood pressure.

Despite the attention paid to elucidating the signalling pathways mediating the physiological actions of NKB, and despite the suggestion that this cytokine might play a role in cardiovascular (dys)regulation during pregnancy, there have been no studies of the effect of chronic administration of NKB, particularly in females. Page et al. had shown that high-dose NKB, infused over 2 h, induces a transient increase in blood pressure in female rats [26]. We determined to develop that study by investigating the effect of chronic infusion of NKB into female rats. We proposed that a modest long-term increase in plasma NKB levels would induce a fall in blood pressure and an attenuated response to pressor agents, which would be mediated through the vasodilatory effects of NKB on the arterial vasculature. By contrast, we proposed that chronic high-dose NKB would induce an increase in systemic blood pressure. We reasoned that the high circulating levels of NKB would cross the blood–brain barrier to induce a centrally mediated pressor response, which would override the direct peripheral vasodilatory activity. We infused low (1.8 nmol/h) or high (20 nmol/h) doses of NKB into conscious female rats. Resting blood pressure, and the pressor responses to phenylephrine (PE) and Ang II were measured telemetrically in conscious rats. Isolated small mesenteric arteries were then mounted in a wire myograph, and concentration–response curves to PE were constructed. As we had hypothesized, the depressor effect of low-dose NKB could be attributed solely to peripheral vasodilation. On the other hand, the pressor effect of high-dose NKB was associated not only with an increase in heart rate (central effect), but also to an *increase* in peripheral vascular tone.

2. Methods

All procedures were approved by the local Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care.

2.1. Animals and housing

Virgin female Long Evans rats weighing 250 to 300 g were obtained from Charles River Canada (St. Foy, Quebec) and housed in a temperature- and humidity-controlled animal facility with a 12:12-h light–dark cycle (6 am/6 pm) for at least 1 week before the experiments. They were maintained on a 0.3% sodium diet and water *ad libitum*.

2.2. Surgery

Surgery was carried out in sterile conditions under sodium pentobarbital anesthesia (62 mg/kg body weight ip) plus atropine (0.1 ml, IM, 0.4 mg/ml). A silastic cannula (0.51-mm ID, 0.94-mm OD; Dow Corning) was implanted non-occlusively into the inferior vena cava (IVC) for drug infusion [18]. A telemetric pressure-recording transmitter (PAC-40, Data Sciences International) was implanted in the abdominal aorta according to the manufacturer's protocol. After surgery, the animals were allowed to recover for 1 week to regain their pre-operative body weight.

2.3. Measurement of hemodynamics

Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored online in the conscious unrestrained rats (PhysioTel Telemetry System, Data Sciences International); data were later analyzed offline (Windaq, DATAQ Instruments). After 1 h of recording baseline MAP and HR, the systemic pressor responses to IV bolus doses of PE (8 μg in 200 μl saline) or Ang II (150 ng in 200 μl saline) were measured (it was determined in preliminary experiments that reproducible maximal pressor responses were obtained at these doses of PE and AII). The data are expressed as the maximal changes in MAP and HR from baseline. Baroreflex gain (△HR/△MAP from baseline) was measured at the height of the pressor response.

2.4. Preparation of isolated mesenteric resistance arteries

The rats were decapitated and a segment (about 10 cm) of the small intestine and attached mesentery was rapidly removed and placed in ice-cold HEPES medium (in mM): 142 NaCl, 4.7 KCL, 1.17 $MgSO_4$, 1.56 $CaCl_2$, 1.18 K_2PO4, 10 HEPES and 5.5 glucose, with a pH of 7.4 [10]. Arterial segments (~2 mm in length, <300 μm in diameter) were dissected free from surrounding adipose tissue. We chose to study the mesenteric bed because it contributes substantially to peripheral vascular resistance in conscious rats [4].

2.5. Small vessel wire myograph

Mesenteric arterial segments were mounted in a wire myograph (Kent Scientific, Litchfield, CA, USA) for isometric tension measurement. After measurement of passive-tension internal circumference characteristics, tension was set to the estimated *in vivo* internal circumference at physiological transmural pressure, as previously described [1,21]. Cumulative concentration–response curves to PE (3×10^{-7} to 5×10^{-5} M) were constructed and the EC_{50} and maximal tension were determined for each vessel. After exposure to PE, acetyl-β-methylcholine chloride (Sigma Chemical) was added to the bath (final concentration 10^{-4} M) to assess the integrity of the vascular endothelium; the vessels were considered acceptable if they exhibited >50% vasorelaxation. At the end of the experiment, potassium chloride-depolarizing solution (125 mM) was added to test the viability of the arteries.