Role of calcium channels and endothelial factors in nickel induced aortic hypercontraction in Wistar rats

Shahnawaz Ahmad WANI, Luqman Ahmad KHAN, Seemi Farhat BASIR

Department of Biosciences, Jamia Millia Islamia, New Delhi 110025, India

Abstract

Aim: To investigate the mechanism of nickel augmented phenylephrine (PE)-induced contraction in isolated segments of Wistar rat aorta. *Materials and Methods*: Effect of varying concentrations of nickel on PE-induced contraction were investigated in isolated segments of Wistar rat aorta using an organ bath system. Aortic rings were pre-incubated with verapamil (1 µM and 20 µM), gadolinium, apocynin, indomethacin or N-G-nitro-L-arginine methyl ester (L- NAME) separately before incubation with nickel. *Results:* Endothelium intact aortic rings incubated with 100 nM, 1 µM or 100 µM of nickel exhibited 80%, 43% and 28% increase in PE-induced contraction, respectively, while no such enhancing responses were observed in endothelium denuded aorta. Incubation of aortic rings with 1 µM and 20 µM verapamil suggested an involvement of influx of calcium through T-type calcium channels in smooth muscle cells, while aortic rings pre-incubated with gadolinium showed no role of store operated calcium channels in the nickel effect on PE-induced contractions. The enhancing effect of nickel on PE-induced contractions was inhibited by apocynin, indomethacin or L-NAME. *Conclusion:* Nickel has caused augmentation of PE-induced contractions as a result of the endothelial generation of reactive oxygen species (ROS) and cyclooxygenase 2 (COX2) dependent endothelium contracting factors (EDCFs), which increases the influx of extracellular calcium through T-type Ca^{2+} channels in smooth muscle cells.

Key words: Nickel, Hypercontraction, L- type Ca^{2+} channels, T-type Ca^{2+} channels, Reactive Oxygen Species

Introduction

Nickel is the fourth most used metal in the world. Its consumption is increasing day by day because of consistent use for many industrial products and medical appliances. In the middle ages, copper miners mistook

Corresponding author: Prof. Seemi Farhat Basir, Ph.D., Department of Biosciences, Jamia Millia Islamia, New Delhi 110025, India
Phone: +91-9810597159 e-mail: sbasir@jmi.ac.in

nickel ore for copper ore and called it kupfer Nickel, "the devil's copper", from which we get its name (1). As per Agency for Toxic Substances and Disease Registry (ATSDR) report, nickel is the 24th most abundant metal with the percentage of nickel within the Earth's core being 6% (2). Nickel is also essential for the function of many microorganisms and is present as a cofactor in many enzymes such as methyl coenzyme M reductase, CO-dehydrogenase, glyoxalase I, Ni-superoxide dismutase, hydrogenase, but when its concentration increases and crosses the limit of essentiality its level may be toxic to living organisms (3–6). Humans are exposed to nickel through natural sources and anthropogenic sources. Natural sources of nickel are weathering of rocks and in soils because of volcanic emissions into the atmosphere. Each year 8.5 million kg of nickel is discharged into the atmosphere (7). Nickel is reported to cause cardiovascular as well as respiratory complications such as high blood pressure and asthma (5). Excessive exposure to nickel is a major cause of vascular diseases, with 11.7% of workers exposed to nickel in China reported to have hypertension (8). It has been estimated that the prevalence of nickel allergy in the general population is 8–15% for females and 1–3% for males (9, 10). Nickel with vanadium can produce a synergistic increase in markers of pulmonary inflammation bronchoalveolar lavage fluid (BALF) as well as potentiated hypothermia, arhythmogenesis and bradycardia (11). Earlier studies have reported that nickel at 10^{-6} M concentration induced vasoconstriction in isolated canine coronary artery due to a tonic calcium activation mechanism and it has been proposed that adrenergic receptors play a role in nickel-induced vasoconstriction (12, 13). Nickel at low concentration (10^{-8}–10^{-7} M) has been shown to contract the dog heart and isolated preparations from the dog heart, as well as inhibition of coronary vasodilation in isolated perfused dog heart (14). Nickel also causes coronary arterial resistance when the coronary vessels are dilated by hypo perfusion, hypoxia and adenosine (15). Nickel has a role in reducing nitric oxide (NO) release from endothelial cells. Das et al. have shown nickel sulphate stimulates inducible nitric oxide synthase (i-NOS) and inhibits endothelial nitric oxide synthase (e-NOS) activities (16). Zhang et al. have reported that nickel leads to rise in expression of cytokines in THP-1 cells (17). Nickel can also produce cytotoxic ROS through TGF-β1 activation (18). Activation of transcription factor NF-κB (nuclear factor κB) is induced by nickel and family of proteins that regulate DNA transcription in cellular response such as inflammatory immune response, cell apoptosis and cell cycle regulation (19–21). Generation of ROS in human bronchial epithelial cell lines (BEAS2B cells) is reported to be enhanced by nickel exposure (22). Endothelial cells are associated with cyclooxygenase (COX) and ROS generation. Therefore, preparations of the aorta which have intact endothelial cells also generate COX and ROS and are reported to cause an increase in calcium through voltage-dependent Ca^{2+} channels (VDCC) as a result of the release of endothelium dependent contraction factors (EDCFs) (23–25). It has been reported that the average concentrations of nickel in serum and urine are 0.2 μgL^{-1} and 0.1 to 13.3 μgL^{-1}(16). Angerer et al. have reported nickel in the urine of welders at a concentration of 18.8 μg L^{-1} (95th percentile 52.5 μgL^{-1}) (26). The purpose of our research was to investigate the acute effect of nickel on isolated segments of the rat aorta at lower concentrations and to elucidate the role of both calcium channels and EDCFs released from the endothelium during nickel-caused hypercontraction of segments of endothelium-intact rat aorta.

Materials and Methods

Animals

Male Wistar rats, 25 in number, weighing 300–500 g, were used in this study as approved by the Institutional Animal Ethical Committee (no. 001/2016), Jamia Millia Islamia, New Delhi, India. They were kept under conditions of constant temperature (27 ± 2 °C) with a standard light/dark cycle (12/12 h) and were fed with standard rat feed and provided drinking water ad libitum. All animals were cared for in compliance

with the Guide for Care and Use of Laboratory Animals, published by the ILAR, National Research Council of the National Academies, USA.

Solutions and Drugs

Phenylephrine (PE), acetylcholine (ACh), apocynin, verapamil, N-G-nitro-L-arginine methyl ester (L-NAME), gadolinium (Gd) and nickel chloride were procured from Sigma Chemicals, St. Louis, USA. Sodium chloride, potassium chloride, magnesium sulphate, dextrose, calcium chloride, potassium dihydrogen phosphate and sodium bicarbonate obtained from Merck (India) were used for preparation of Krebs buffer with composition (in mM): 120 NaCl; 25 $NaHCO_3$; 1.2 $MgSO_4$; 1.2 KH_2PO_4; 4.72 KCl; 2.5; $CaCl_2$ and 11 $C_6H_{12}O_6$.

Measurement of aortic contractile activity

Wistar rats were anesthetized with pentobarbital (30 mg/kg body weight) (27). The thoracic aorta of each rat was removed and dipped either in cold Krebs buffer or in Krebs buffer without Ca^{2+} at room temperature. White fat covering the aorta was removed manually, and each aorta was cut transversally into 4–5 mm circular rings with care to avoid any damage to the endothelium. Rings were mounted between two stainless steel wires in 15 mL organ baths containing Krebs medium continuously bubbled with 95% O_2 and 5% CO_2 at 37°C. All experiments were performed after an equilibration period of 60 min with bathing medium renewed after every 15 min, which ruled out trauma or any other extraneous effects. Endothelium-intact aortic rings were stretched with a passive tension of 2.0 g. The tension was recorded using an isometric force transducer (MLT0420, AD Instruments, Australia) connected to a PC-based Data acquisition system from AD Instruments (PL3508 Power-Lab 8/35). Control contractions were induced with 1 µM PE to get maximum response at this concentration (Fig. 1A) (28). Each aortic preparation was challenged at the beginning of the experiment with 1 µM of ACh, and if the vaso-relaxant response to ACh was greater than 50% of the PE-induced contraction, the aortic segment was considered to possess an intact endothelium.

Different concentrations of nickel (100nM, 1µM, and 100 µM) were added to Krebs buffer to study nickel induced hypercontraction (29). Aortic rings were incubated for 40 min with each concentration of nickel and their response to 1 µM PE recorded. To investigate the mechanism of the effects of nickel, the endothelium-intact aortic rings were first pre-incubated with either 1 µM verapamil (L-type calcium channel blocker), 20 µM verapamil (L-type and T-type calcium channel blocker) and 10 µM Gadolinium (store operated calcium channel blocker), 100 µM apocynin (NADPH oxidase inhibitor), 100 µM indomethacin (non-selective COX inhibitor) or 100 µM L-NAME (eNOS inhibitor) separately for 40 min and followed by incubation with nickel for another 40 min before the PE responses were examined.

Statistical analyses

Data in our study are represented as the mean ± S.E.M. "n" represents the total number of experiments, and all the experiments were performed on aortic rings taken from different animals. ANOVA and un-paired student's t test were done for statistical analysis wherever applicable. $P \le 0.05$ was considered to be statistically significant data.

Results

Effect of nickel on PE-induced contraction of rat aortic rings

Incubation with nickel (100 nM, 1 µM and 100 µM) caused an increase in PE-induced contraction in endothelium intact aortic rings from male Wistar rats. Nickel modulated the PE-induced contraction and caused
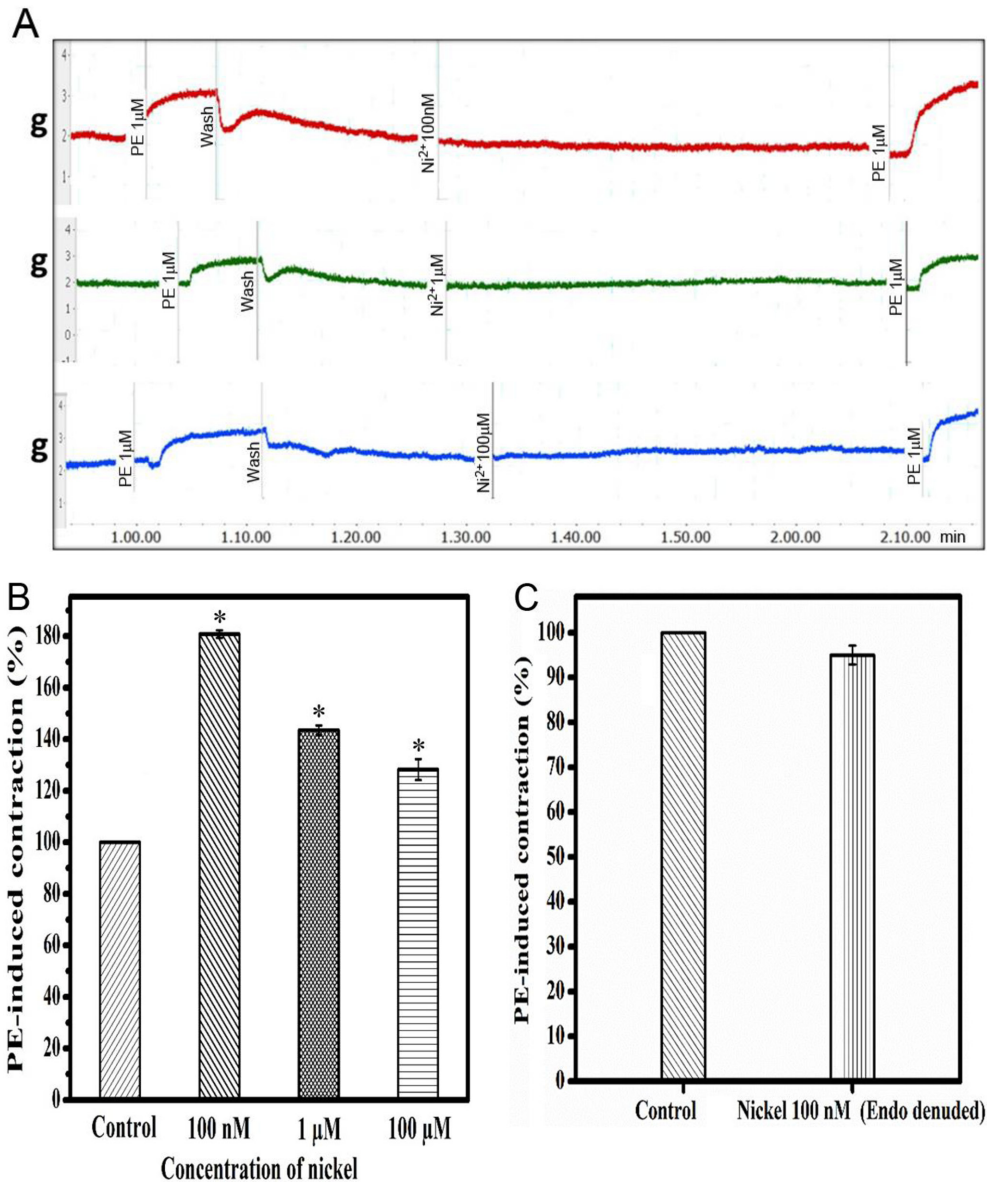
Fig. 1. Effect of nickel on PE-induced contraction. A: Representative traces. Nickel concentrations applied were 100 nM (red), 1μM (green) and 100 μM (blue). B: Effect of three concentrations of nickel (100 nM, 1 μM and 100 μM) on PE-induced contraction in the endothelium intact aortic rings. Contractions are expressed as the percentage of the control in each individual experiment. Values represent the mean ± S.E.M. (*n*=10). *$P \leq 0.05$ versus control. C: Effect of nickel (100 nM) on PE-induced contraction in the endothelium denuded aortic rings. Contractions are expressed as the percentage of the control in each individual experiment. Values represent the mean ± S.E.M. (*n*=5). There was no significant difference between effects of nickel exposed and unexposed rings.

180 ± 1.51% contraction at 100 nM concentration and at 1μM concentration a 143.5 ± 1.86% contraction, while at 100 μM concentration of nickel the contractile response was 128 ± 4.1% with respect to the control as shown in Figs. 1A and B. As the largest effect was obtained at 100 nM, this concentration of nickel was used from here on. Fig. 1A also shows that nickel alone (in the absence of PE) did not show any effect in the tension recording. Aortic rings which were denuded of endothelium did not show any augmentation in contraction at 100 nM concentration of nickel as compared to endothelium intact aortic rings as shown in Fig. 1C.
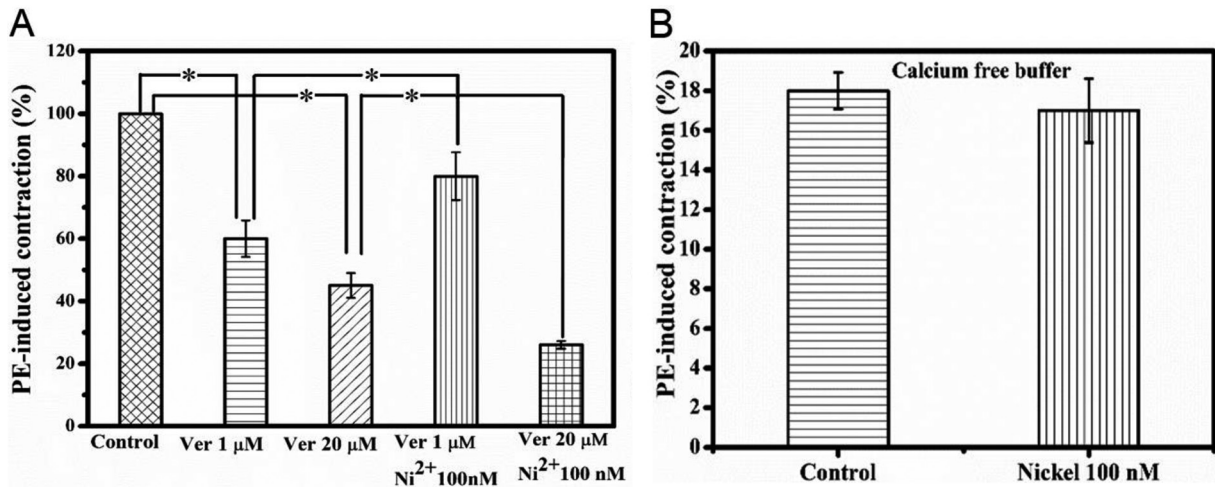
Fig. 2. A: Effect of verapamil (1 and 20 μM) on nickel exposed and unexposed rings. Contractions are expressed as percentages of the control (in 2.5 mM Ca^{2+}) in each individual experiment. Values represent the mean ± S.E.M. ($n=5$); *$P \leq 0.05$ (ANOVA followed by Duncan's multiple range test). B: Effect of nickel in calcium free buffer. Contractions are expressed as percentages of the control in each individual experiment. Values represent the mean ± S.E.M. ($n=5$). There was no significant difference between effects of nickel exposed and unexposed rings.

Role of nickel in stimulation of L-type and T-type calcium channels of the PE-induced contraction of rat aortic rings

We observed that nickel increased the percentage contraction irrespective of the presence or absence of 1 μM verapamil (Fig. 2A). Verapamil 1 μM alone caused 40 ± 5.83% inhibition, while pre-incubating aortic rings with 1 μM verapamil for 40 min and then with 100 nM nickel for 40 min, the decrease was only 20 ± 7.61% with respect to the control. Verapamil at this concentration is only able to inhibit L-type Ca^{2+} channels. Further, to block both L-type and T-type calcium channels, isolated aortic rings were incubated with 20 μM verapamil. Verapamil at 20 μM concentration caused 56 ± 3.96% inhibition in unexposed endothelium-intact aortic rings as compared to the control. Pre-incubation of aortic rings with 20 μM verapamil for 40 min followed by nickel for 40 min has increased the percentage of inhibition up to 74 ± 0.2%. Moreover, to check the role of intracellular calcium in the effect of nickel, experiments were conducted in calcium-free buffer. When aortic rings were pre-contracted with PE in calcium-free buffer and then incubated with nickel for 40 min followed by contraction with PE again, that caused only about 18% and 17% contraction in unexposed (i.e. control) and in nickel exposed endothelium intact aortic rings, respectively, as shown in Fig. 2B.

Effect of nickel on extracellular CaCl$_2$ induced contraction in rat aortic rings

We observed a rise in contraction when $CaCl_2$ (0.25 to 2.5 mM) was added cumulatively to aortic rings exposed to nickel as compared to the control rings, as shown in Fig. 3A. Aortic rings were incubated with nickel for 40 min, 1 μM PE was applied and then 0.25 to 2.5 mM $CaCl_2$ was added cumulatively at 10 min intervals. Nickel enhanced the Ca^{2+}-induced contraction in the presence of PE. In another series of experiments aortic rings were incubated with 1 μM verapamil for 40 min followed by contraction through cumulative addition of $CaCl_2$ and the effect on nickel contractions was examined (Fig. 3B). The results showed that $CaCl_2$ was not able to maximise contraction in the presence of verapamil with respect to the control but after incubating aortic rings with 1 μM verapamil, 100 nM nickel enhanced the contraction. On incubating aortic rings with 20 μM verapamil in one series of experiments, we observed that there was miniscule enhancement in percentage contraction by cumulative addition of the $CaCl_2$ (Fig. 3C).
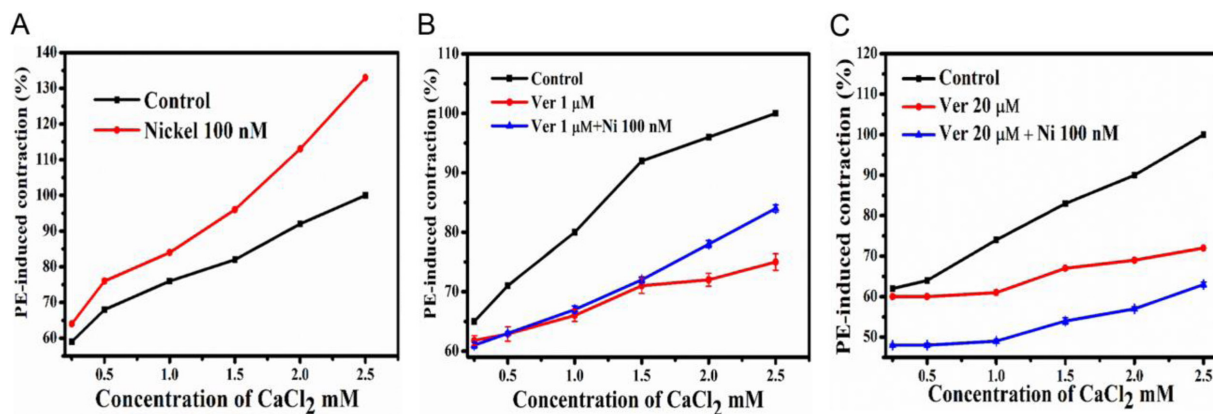
Fig. 3. Ca^{2+} induced contraction in the presence of 1 μM PE. Contractions are expressed as percentages of the contraction at 2.5 mM $CaCl_2$ in each individual experiment. A: Effect of Ni^{2+} (100 nM). B: Effect of Ni^{2+} (100 nM) in the presence and absence of verapamil (1 μM). C: Effect of Ni^{2+} (100 nM) in the presence and absence of verapamil (20 μM). Values represent the mean ± S.E.M. (*n*=6).
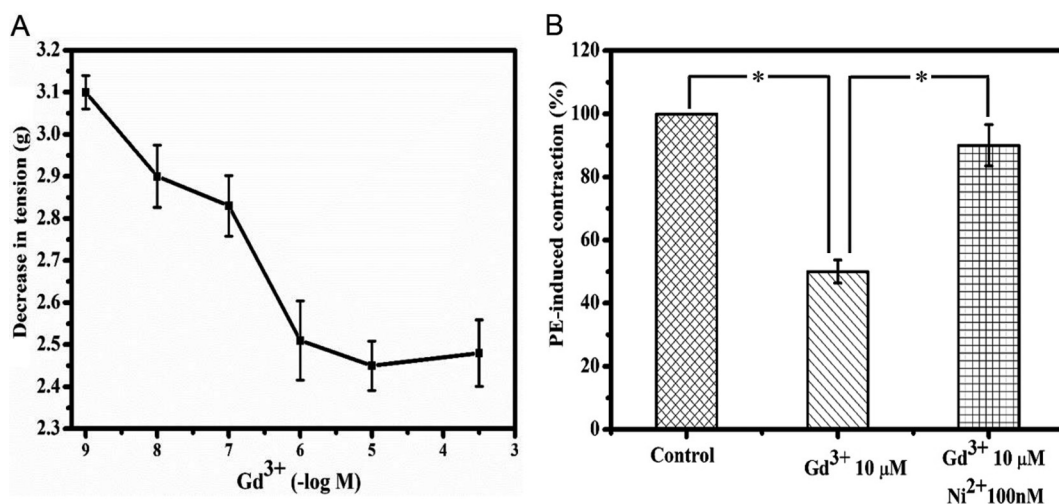

Fig. 4. A: Cumulative concentration response curve of gadolinium (Gd^{3+}) on PE-induced contraction in isolated aortic rings Values represent the mean ± S.E.M. (*n*=5). B: Effect of gadolinium 10 μM on nickel exposed and unexposed aortic rings. Contractions are expressed as percentages of the control in each individual experiment. Values represent the mean ± S.E.M. (*n*=10); *$P \leq 0.05$ (ANOVA followed by Duncan's multiple range test).

Effect of a store operated Ca^{2+} channels (SOCC) inhibitor on nickel augmented contraction over PE-induced contraction of rat aortic rings

We observed that Gd^{3+} caused dose-dependent inhibition of the PE-induced contraction (Fig. 4A). A 10 μM concentration of Gd^{3+} was later selected for inhibiting SOCC from here on. In Fig. 4B, aortic rings exposed to Gd^{3+} (10 μM) caused a 50 ± 3.66% decrease in the percentage of inhibition while only a 10 ± 6.53% decrease in percentage of inhibition was noticed in the case of aortic rings incubated with 10 μM Gd^{3+} + 100 nM nickel as compared to the control.

Effect of apocynin, indomethacin or L- NAME on nickel augmented contraction over PE-induced contraction of rat aortic rings

NADPH oxidase inhibitor (apocynin) caused a 38 ± 4.59% inhibition of contraction in nickel un-exposed aortic rings, and 60 ± 3.9% of inhibition in nickel exposed aortic rings (Fig. 5A). We noticed a 45 ± 2.78%
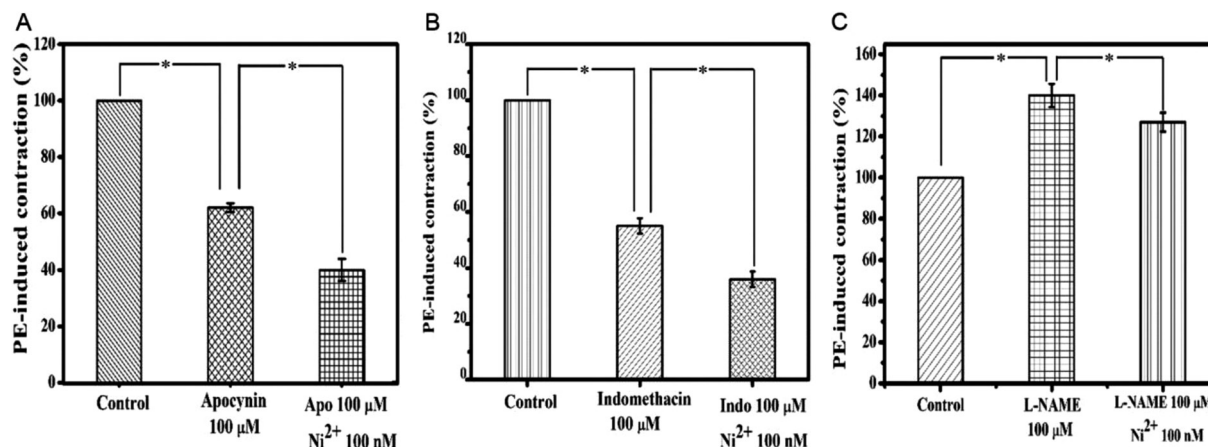
Fig. 5. A: Effect of nickel (100 nM) on PE-induced contraction in the presence of apocynin (100 μM). Contractions are expressed as percentages of the control in each individual experiment. Values represent the mean ± S.E.M. (n=15); * $P{\leq}0.05$ (ANOVA followed by Duncan's multiple range test). B: Effect of nickel (100 nM) on PE-induced contraction in the presence of indomethacin (100 μM). Contractions are expressed as percentages of the control in each individual experiment. Values represent the mean ± S.E.M. (n=6); *$P{\leq}0.05$ (ANOVA followed by Duncan's multiple range test). C: Effect of nickel (100 nM) on PE-induced contraction in the presence of L-NAME (100 μM). Contractions are expressed as percentages of the control in each individual experiment. Values represent the mean ± S.E.M. (n=10); *$P{\leq}0.05$ (ANOVA followed by Duncan's multiple range test).

inhibition after incubation of aortic rings with 100 μM indomethacin (a non-selective COX inhibitor). Incubation of aortic rings with both indomethacin and nickel caused an increase in the percentage of inhibition to 64 ± 2.79 (Fig. 5B). In aortic rings exposed to 100 μM of L-NAME (a potent inhibitor of nitric oxide synthase), PE-induced contraction increased by 40 ± 5.7% in unexposed aortic rings; however, only a 27 ± 4.6% increase in contraction was observed in L-NAME and nickel exposed aortic rings as shown in Fig. 5C.

Discussion

It has been reported that acute exposure to nickel at 10^{-7} M concentration had caused hypercontraction in uterine strips and cardiac muscle (12, 30, 31); the reason for the rise in hypercontraction was reportedly due to uptake of extracellular Ca^{2+} through VDCC (30). Other studies have reported that nickel at higher concentrations caused blockage of Ca^{2+} channels (32) and therefore is used as a Ca^{2+} channel blocker (33). In our study, we examined the PE-induced contraction of the endothelium-intact isolated Wistar rat aortic rings in the presence of three different concentrations (100 nM, 1 μM and 100 μM) of nickel. Nickel exhibited an enhancing effect of PE-induced contraction at all three concentrations with the largest effect at the lowest concentration and the smallest at the highest concentration examined. Nickel at 500 μM caused a decrease in PE-induced contraction below the control level (data not shown), as has also been reported by Hobai et al. (33). Nickel exhibits a dose-dependent effect, with the enhancing effect on contraction starting at lower concentrations and the inhibiting effect at higher concentrations. Since the highest contraction in endothelium intact aorta segments was seen at 100 nM concentration, we performed the rest of our experiments using this concentration of nickel to determine the mechanism of hypercontraction. In the case of endothelium denuded Wistar rat aortic rings, no nickel augmented hypercontraction was observed on the PE-induced contraction (Fig. 1C); this is inconsistent with the results of Evans et al. (34) while in agreement with the results of Liu et al. (35).

To investigate the role of VDCC in nickel augmented PE-induced vasoconstriction, endothelium-intact

aortic rings were treated with 1 µM verapamil (L-type Ca^{2+} channel blocker). We observed $40 \pm 5.83\%$ of inhibition as compared to control. When aortic rings were pre-incubated with 1 µM verapamil followed by 100 nM nickel, we observed a $20 \pm 7.61\%$ of inhibition. It has been reported that nickel caused activation of T-type Ca^{2+} channels which is why in the presence of 1 µM verapamil there was a rise in PE-induced contraction (13). T-type Ca^{2+} channels are reported to be present in aortic smooth muscle cells and cardiac muscle cells. According to Golenhofen, two chemically different systems for Ca^{2+} activation exist in smooth muscle cells, which are P-type and T-type channels. P-type channels are responsible for phasic contraction while T-type Ca^{2+} channels are responsible for tonic contraction (36). In order to validate the role of T-type Ca^{2+} channels in hypercontraction, endothelium-intact aortic rings were treated with 20 µM verapamil which blocks both L-type and T-type Ca^{2+} channels (37). On incubation of aortic rings with 20 µM verapamil, we observed a $56 \pm 3.96\%$ inhibition in nickel unexposed aortic rings and a $74 \pm 0.2\%$ inhibition in nickel exposed aortic rings as shown in Fig. 2A. Our results in Fig. 2A suggest that the hypercontraction may be due to the influx of extracellular Ca^{2+} via T-type Ca^{2+} channels. Rubanyi et al. have also reported that a rise in basal tone was due to activation of T-type Ca^{2+} channels (13). In the absence of extracellular calcium, PE caused phasic contraction by releasing intracellular calcium from endoplasmic reticulum (38). We too observed a small rise in contraction but the presence of nickel in calcium free buffer has not enhanced the constriction of the aortic segments further (Fig. 2B). Similar results were reported by Rubanyi et al. (13).

We further investigated the role of nickel in activation of both L-type and T-type channels by cumulative addition of $CaCl_2$ (0.25 to 2.5 mM) in the presence of 1 µM PE. Our results showed that the $CaCl_2$ induced contractions in aortic rings were enhanced by nickel with respect to nickel unexposed aortic rings (Fig. 3A). It was also true in the presence of 1 µM verapamil (Fig. 3B). Previous studies have also reported that verapamil acts as a selective Ca^{2+} antagonist, which reduces nickel-induced coronary vasocontraction both in the *in situ* dog heart and the isolated rat heart (31, 39). In addition, step-wise elevation of Ca^{2+} increases the amplitude of contraction in nickel-exposed tissue in presence of verapamil (13). We noticed a significant decrease in contraction when aortic rings were pre-incubated with 20 µM verapamil and then followed by incubation with 100 nM nickel, further validating our results that T-type Ca^{2+} channels may be responsible for influx of extracellular calcium due to the presence of nickel (Fig. 3C). Trivalent cations like gadolinium and lanthanides are reported to be potent Ca^{2+} channel blockers; and are believed to displace Ca^{2+} from binding sites on its external surface of the plasma membrane thereby inhibiting Ca^{2+} ion influx and efflux (40). Gd^{3+} is particularly effective and is an irreversible Ca^{2+} channel blocker at micro molar concentration (41). Gd^{3+} has been reported to cause inhibition of SOCC at concentrations of from 1 µM to 5 µM (42–44). It has been reported to cause inhibition of stretch-activated channels at a concentration of 10 µM (45). SOCC are activated when the Ca^{2+} level in the endoplasmic reticulum falls, after which STIM1 (stromal interacting molecule 1) present on the endoplasmic surface activates ORAI channels present in the plasma membrane to influx extracellular Ca^{2+} (46, 47). Our results also show that PE-induced contractions were very much inhibited by 10 µM Gd^{3+} (Fig. 4A). Gd^{3+} is also reported to be an L-type Ca^{2+} channel blocker at micromolar concentrations (48). Hence in the presence of 10 µM Gd^{3+} blocking both SOCC and L-type Ca^{2+} channels, we observed a significantly greater rise in contraction in nickel exposed aortic rings than in nickel unexposed aortic rings (Fig. 4B). This therefore negates a role for SOCC in hypercontraction.

To investigate nickel augmented hypercontraction further, endothelium intact aortic rings were pre-incubated with the NADPH oxidase inhibitor (apocynin) and the non-selective COX inhibitor (indomethacin) separately then followed by incubation with 100 nM nickel. The contractile effect of nickel may be due to generation of ROS in the endothelium as shown in Fig. 5A; the nickel unexposed aortic rings have shown a

38 ± 4.59% inhibition of contraction, and a 60 ± 3.9% of inhibition in nickel exposed tissue in the presence of apocynin. Nickel induced production of ROS is also reported in various tissues like epithelial-mesenchymal cells (49). Xi et al. have reported that nickel is an active inducer of ROS in intact mammalian cells and that nickel carcinogenesis may involve multiple types of oxidative damage (50). Aortic rings were also incubated with 100 µM indomethacin alone and we observed a 45 ± 2.78% inhibition of contraction. Pre-incubation of aortic rings with indomethacin, followed by nickel, caused a further inhibition of PE induced contraction of 64 ± 2.79% as shown in Fig. 5B. Relaxation shown by nickel-exposed aortic rings in the presence of apocynin or indomethacin was more than that in nickel un-exposed aortic rings, indicating that hypercontraction induced by nickel has a significant contribution from pathways involving ROS and COX. ROS is also reported to be an EDCF (51). Nickel is reported to cause COX2 expression in human bronchial epithelial cell line (Beas-2B) cells (52) by enhancing expression of the transcription factor NF-κB. Nickel also causes expression of inflammatory mediators like TNF α, IL6, IL8 and COX2 (53). Thus, NADPH oxidase derived ROS and some of the EDCFs released from COX2 pathway induce influx of extracellular calcium into the smooth muscle cells through T-type channels and may be responsible for the hypercontraction by nickel (54). Gorlach et al. have reported the mutual interconnection between production of ROS and calcium release, and have reported that ROS significantly affects the influx of calcium both into the cell and into intracellular calcium stores (55).

Hypertension and atherosclerosis are pathological conditions that are responsible for reduction of NO bioavailability (56). ROS are reported to cause reduction of NO, and NO reduction is reported to increase vascular reactivity inducing vasoconstriction (57). In our study, aortic rings were exposed with a potent inhibitor of eNOS (L-NAME 100 µM) that caused a 40 ± 5.7% increase in contraction in nickel unexposed aortic rings. This result indicates that there is some basal release of NO which suppresses PE-induced contraction. Once NO production is blocked by L-NAME the contraction increases in the absence of NO. In presence of L-NAME, nickel did not enhance PE-induced contraction but rather suppressed it, indicating that nickel may suppress the basal release of NO and enhance PE-induced contraction. As ROS are reported to cause reduction of NO (57), nickel may induce endothelial production of ROS which in turn reduced the NO production enhancing PE-induced contraction. The suppression of contraction in the presence of L-NAME may be due to the calcium channel blocking effect of nickel.

Conclusion

From this study we have concluded that the nickel-induced hypercontraction to PE is due to the endothelial generation of ROS derived from NADPH oxidase and due to the endothelial release of hypercontractile prostanoids, derived from the COX-2 pathway. Reduction of endothelial NO release may also be involved. And the presence of ROS and prostanoids may be responsible for influx of Ca^{2+} through T-type Ca^{2+} channels to smooth muscle cells. We have also negated the role of L-type calcium channels and SOCC in hypercontraction of aorta in the presence of nickel. Therefore, acute exposure to nickel can contribute to an increased vascular resistance, and subsequently to the beginning and continuation of hypertension.

Acknowledgment

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