Inhibitory effect of manganese on contraction of isolated rat aorta

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The effects of MnCl$_2$ on vascular smooth muscle contraction induced by noradrenaline (NA) and KCl were investigated. Rings segments from rat aorta were isolated and changes in isometric tension recorded. MnCl$_2$ (10 $\mu$M and 1 mM) significantly attenuated the contractile responses to NA and KCl. There were also reductions in the contractile responses to CaCl$_2$ in NA- and KCl-stimulated rings, after pretreatment with MnCl$_2$. The magnitude of the phasic contraction to NA was significantly reduced in presence of MnCl$_2$. The results suggest that MnCl$_2$ inhibits vascular smooth muscle contraction by influencing a Ca$^{2+}$-mediated mechanism.

Keywords: Calcium, MnCl$_2$, Vascular contraction

The use of Mn$^{2+}$ when complexed to DPDP (N,N'-dipryidoxylethenediamine-N,N'-diacetate-5,5'-bisphosphate) as an MRI contrast agent is usually accompanied by vascular changes, notably peripheral vasodilation$^1$. Similar effects have been reported with MnCl$_2$ both in whole animals and isolated vascular preparations$^2$. Asplund et al.$^3$ reported a direct endothelium-dependent relaxant effect of Mn$^{2+}$, and Matsunaga and Furchgott$^4$ showed a potentiation of the vasodilatory effect of exogenous nitric oxide in endothelium-denuded vessels. These effects have been attributed to the protection of nitric oxide from inactivation by superoxide radical. The possibility of the involvement of other mechanisms in the action of Mn$^{2+}$ on vessels deserves investigation. The excitation-contraction coupling in vascular smooth muscle cells is dependent on extracellular Ca$^{2+}$ influx and intracellular Ca$^{2+}$ mobilization$^5,6$. Some reports suggest that Mn$^{2+}$ may inhibit influx of extracellular Ca$^{2+}$ in cardiac$^7$ and vascular smooth muscle cells$^8$. The present study has been designed to investigate the effect of Mn$^{2+}$ on noradrenaline- and KCl-induced contractions which depend on the influx of Ca$^{2+}$ via receptor-operated and voltage-dependent channels respectively, and the possibility that its effects may also involve intracellular Ca$^{2+}$ mobilization.

Materials and Methods

Tissue preparation. — The thoracic aorta was isolated from 12-15 week old Sprague-Dawley rats and cut into rings of about 3 mm in length, each of which was suspended in organ bath containing Krebs-Henseleit buffer of the following composition (mM): NaCl 119, KCl 4.7, KH$_2$P$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 14.9, CaCl$_2$ 1.6, glucose 11.5. The solution was continuously bubbled with 95% O$_2$:5% CO$_2$ gas mixture at 37°C and pH 7.4. Tension was measured isometrically using a force-displacement transducer (FT 03C) connected to a Grass Model 7D polygraph. Tissues were allowed to equilibrate for 90 min under a resting tension of 2 g prior to the start of experiments. All experiments were performed using endothelium-intact aortic rings.

Concentration-response test to NA and KCl — After the equilibration period, cumulative concentration-response tests to NA (1 nM to 10 $\mu$M; Sigma, UK) and KCl (10 to 80 mM) were carried out in normal aortic rings, with the higher concentration being added only after the effect of the previous one had reached a plateau. After a 30 min equilibration period, the tissues were treated with MnCl$_2$ (10 $\mu$M or 1 mM) for 15 min and the concentration-response tests to NA and KCl repeated. Concentration-response tests to KCl were carried out using Krebs-Henseleit buffer prepared by equimolar substitution of NaCl with KCl.

Concentration-response test to CaCl$_2$. — In order to ascertain the effect of Mn$^{2+}$ on extracellular Ca$^{2+}$ influx, the concentration-response test to CaCl$_2$ was conducted in presence of NA or KCl. Briefly, the procedure was as follows: Freshly equilibrated rings were contracted with NA (10 $\mu$M) and at the peak of contraction, the tissues were rinsed with Ca$^{2+}$-free Krebs-Henseleit buffer containing EGTA (1 mM).
After 15 min the tissues were again treated with NA (10 µM) and rinsed with Ca²⁺-free Krebs-Henseleit buffer containing EGTA (1 mM) to ensure complete depletion of NA-sensitive intracellular Ca²⁺ stores. After 15 min, the bathing medium was replaced with Ca²⁺-free Krebs-Henseleit buffer without EGTA but containing either NA (10 µM) or KCl (80 mM). The rings were maintained for 10 min in either of these solutions with or without MnCl₂ (10 µM or 1 mM) present, before commencement of the cumulative addition of CaCl₂ (10 µM-10 mM).

Intracellular Ca²⁺ mobilization — After equilibration, aortic rings were first contracted with NA (10 µM) and at the peak of contraction rinsed in normal Krebs-Henseleit buffer and allowed to equilibrate for 30 min. The tissues were then exposed to Ca²⁺-free Krebs-Henseleit buffer containing EGTA (1 mM) for 15 min before treatment with NA (10 µM). The resulting phasic contractile response is a measure of the release of intracellular Ca²⁺ from noradrenaline-sensitive stores⁹. The magnitude of the phasic response was expressed as a percentage of the initial tension in response to NA.

Statistical analysis — Comparison between means was done either by Student’s t-test or by one-way ANOVA followed by Bonferroni’s test. The EC₅₀ values (concentration producing 50% of the maximal response) were calculated by regression analysis.

Results
Effect of Mn²⁺ on NA- and K⁺-mediated contraction — The baseline tension in aortic rings was not altered in presence of 10 µM or 1 mM MnCl₂. The concentration-response curves to NA and KCl in presence of 10 µM and 1 mM MnCl₂ were shifted to the right of the control curve (Fig. 1). The EC₅₀ values for NA and KCl after MnCl₂ treatment were significantly higher than that in the control. Also, the maximal response to both of these agents was significantly attenuated by MnCl₂ (Table 1).

Effect of Mn²⁺ on extracellular Ca²⁺ influx — The concentration-response curves to CaCl₂, developed in Ca²⁺-free Krebs-Henseleit buffer containing 10 µM NA or 80 mM KCl, were shifted to the right, and the maximal responses were significantly (P<0.05) reduced in presence of MnCl₂ (10 µM and 1 mM). The effect of MnCl₂ was dose-dependent with the greater inhibition observed in both cases at 1 mM MnCl₂ (Fig. 2).

Discussion
The vasorelaxant effect of Mn²⁺ on vascular smooth muscle is well known⁵, and MnCl₂ is frequently used as a superoxide dismutase mimetic⁹,¹⁰. The ability of Mn²⁺ to cause direct endothelium-dependent vascular
It is well established that the free Ca\textsuperscript{2+} necessary for excitation-contraction coupling in vascular smooth muscle is of dual origin: influx from the extracellular medium and the release from intracellular stores\textsuperscript{5,6}. Further, Mn\textsuperscript{2+} has been shown to enter vascular smooth muscle cells\textsuperscript{7}, and may therefore modulate signal transduction processes resulting in inhibition of Ca\textsuperscript{2+} mobilization from intracellular stores. In this study, pretreatment with Mn\textsuperscript{2+} in absence of extracellular calcium (due to the presence of EGTA) led to a reduction in the phasic contractile response to NA, possibly by inhibition of release of Ca\textsuperscript{2+} from NA-sensitive intracellular stores.

In conclusion, the results of the present study suggest that Mn\textsuperscript{2+} reduces the maximal response and sensitivity of vascular smooth muscle to contractile stimuli by influencing Ca\textsuperscript{2+-} associated mechanisms.

References