

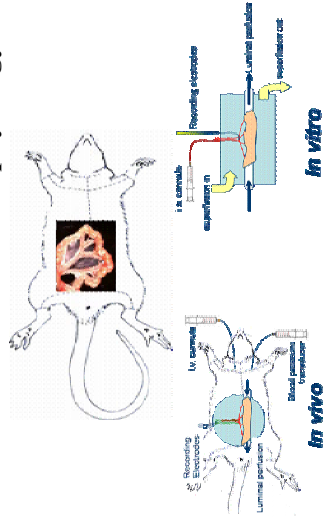
Introduction

We have previously reported that *Nippostrongylus brasiliensis* (Nb) antigen activates mesenteric nerves. In an attempt to elucidate the mechanism of this Nb antigen-induced activation, the mast cell stabilizer doxantrazole was employed. This report describes initial studies with doxantrazole (DOX).

Methods

In order to confirm doxantrazole as a mast cell stabilizer, cultured RBL-2H3 cells and bone marrow-derived mast cells (BMMC) were exposed to ionomycin (1 μ M) and doxantrazole (1 mM, GSK) and the supernatant collected for ELISA analysis of TNF- α and histamine. After the mast cell stabilizing properties of doxantrazole were proved by the above experiments. Mesenteric afferent recordings were obtained both *in vivo* from anaesthetized rats (n=12) and *in vitro* from rat jejunal segments (n=5) using conventional extracellular recording techniques (see the schematic illustration below). Doxantrazole was administered *in vivo* (n=6, 10 mg/kg i.v.) and close intra-arterially *in vitro* (n=5, 0.63 μ g). Cromolyn was administered *in vivo* (n=6, 20 mg/kg i.v.). Data are expressed as mean \pm sem and analysed with One-way ANOVA or repeated-measures one-way ANOVA followed by appropriate post test.

Extracellular electrophysiology



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Results

A: Histamine release from BMMC B: TNF α release from BMMC

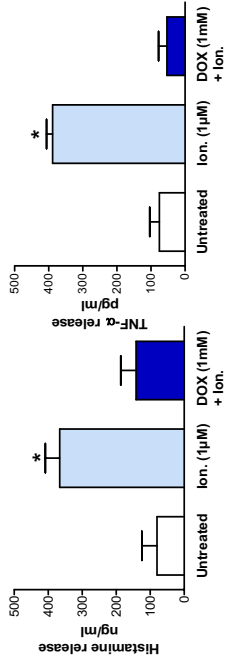


Figure 1 - Effects of doxantrazole on histamine and TNF α release-evoked by ionomycin in BMMC. Doxantrazole inhibited the ionomycin (Ion.)-induced release of both histamine ($P<0.05$, n=3) and TNF α ($P<0.05$, n=3). * $P<0.05$ vs another two groups.

A: IgE-induced TNF α release from RBL cells

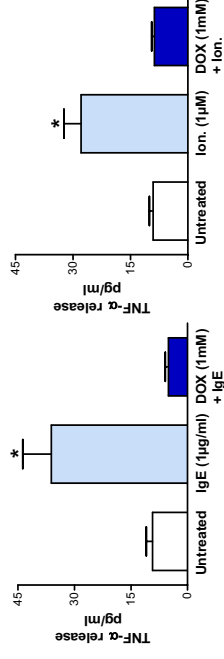


Figure 2 - Effects of doxantrazole on IgE or ionomycin-evoked TNF α release from RBL-2H3 cells. IgE or ionomycin increased TNF α release to a significantly level ($P<0.05$ vs untreated, n=3), but pretreatment with doxantrazole abolished this response ($P<0.05$, n=3). * $P<0.05$ vs another two groups.

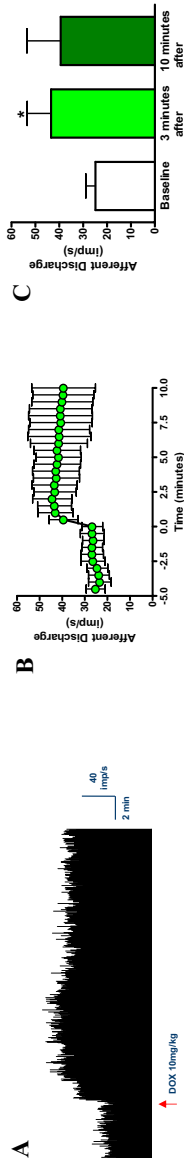


Figure 3 - Doxantrazole activated rat mesenteric afferent nerves *in vivo*. Doxantrazole evoked a consistent and sustained afferent nerve activation with a short response delay time (7.5 ± 0.6 s). A) A sample data showing the alteration in afferent nerve discharge rates after doxantrazole administration. B) Group data of the nerve response, n=6. C) The nerve discharge remained elevated 10 min after DOX administration. * $P<0.05$ vs baseline.

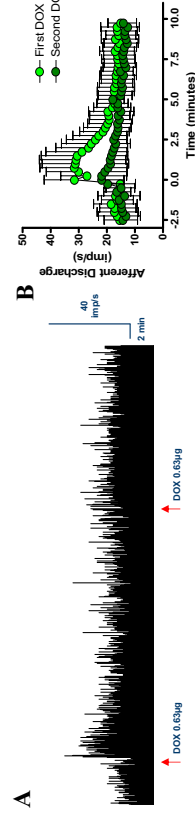


Figure 4 - Doxantrazole activated rat mesenteric afferent nerves *in vitro*. Doxantrazole caused a transient activation of mesenteric nerve and this response desensitized as the second response after 15 min interval was smaller and there was no recordable third response (not shown on this figure). A) A sample data showing the alteration in afferent nerve discharge rates after doxantrazole administration (i.a.). B) Group data of the nerve response, n=5. C) Peak nerve response to doxantrazole compared with baseline before injection. * $P<0.05$ vs baseline before.

Conclusions

- The mast cell stabilizing properties of doxantrazole were confirmed by the inhibition of mast cell mediators release in culture.
- Doxantrazole elicits a sustained increase in afferent nerve firing *in vivo* and this response is mimicked by another mast cell stabilizer cromolyn.
- The nerve response to doxantrazole *in vitro* differs as it is transient and desensitizes.
- The activating property of doxantrazole on afferent nerves may partially explain the lack of efficacy of it as an anti-allergenic agent.