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Compound 48:80 induction of histamine and NO release from isolated rat colon

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Introduction: Compound 48/80 (C48/80) is a polymer of N-methyl-p-methoxyphenethylamine crosslinked by formaldehyde, and has been used to induce histamine release from mast cells through degranulation, primarily through interaction with MAS-related G protein-coupled receptor-X2 (1). Mast cells act as sentinel cells within the gastrointestinal tract, and when stimulated through differing receptor types produce a range of inflammatory mediators and cytokines, including nitric oxide (2). In addition, there is evidence to suggest that ageing influences the degranulation response of mast cells (3). The aim of this study was to assess the effect of "age" on histamine and NO release from the colon of rats in response to C48/80 stimulation. The mast cell stabiliser and H_1 receptor agonist ketotifen was used to evaluate the contribution of mast cells to mediator release.

Method: Tissues were obtained from 6 adult ex-breeding female (~450g) and 4 juvenile (~150g) Wistar rats. Full-thickness strips of colon were dissected and individual pieces were placed in tissue wells containing Dulbecco's modified eagle medium pre-prepared with one of the following: (a) control (b) 10µg/ml C48/80; (c) mast cell stabiliser and H_1 receptor agonist ketotifen (1µM); or (d) C48/80 + ketotifen. The tissues were gassed with 95% O_2 /5% CO_2, and maintained at 37°C for 20 minutes. Subsequently the supernatant was removed and tissues were blotted and weighed. Supernatant histamine was measured using a direct ELISA and NO was assayed using the Greiss reaction. Data are given as mean ± SEM, and were analysed using 2-way and 1-way ANOVA followed by Dunn's multiple comparison post-hoc tests (GraphPad Prism v6).

Results:

		Control	C48/80	Ketotifen	C48/80 + Ketotifen
Histamine $ng.mg^{-1}$	Juvenile	0.15 ± 0.13	0.43 ± 0.38	0.29 ± 0.26	0.25 ± 0.22
	Adult	0.15 ± 0.13	0.70 ± 0.25	0.19 ± 0.12	0.15 ± 0.12
	Merged	0.15 ± 0.09^{bd}	0.59 ± 0.2^{c}	0.23 ± 0.12^{bd}	0.19 ± 0.11^{c}
NO $nM.mg^{-1}$	Juvenile	2.6 ± 0.8^{a}	26.1 ± 15.9^{a}	16.3 ± 9.3	3.7 ± 2.4
	Adult	5.7 ± 2.4	14.7 ± 0.3^{b}	7.6 ± 1.6	2.2 ± 1.3^{b}
	Merged	4.4 ± 1.5^{b}	19.3 ± 6.4^{bc}	11.1 ± 3.8	2.8 ± 1.2^{c}

Results on the same row with the same superscript differ significantly (a: P< 0.05; b: P< 0.01; c: P< 0.001; d: P<0.0001)

Conclusion: In summary, the response profile is similar between both age groups, and there was no significant effect of "age" seen in this study. C48/80 induced both histamine and NO release which was attenuated in the presence of the mast cell stabiliser ketotifen. These effects may be mediated through direct action of C48/80 on tissue mast cells in colon from both young and adult rats.

References:

1. Hoffmann HJ (2015) *Int Arch Allergy Immunol*. **168**:253-62.
2. Moon T *et al*. (2014) *Front Immunol* **5**: 569.
3. Nguyen M *et al* (2005). *J Immunol* **175**: 5701-5707.