

## Contribution Of TRPA1 Agonist Actions Of Methylglyoxal To Colon Motility Dysfunction In Diabetes

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**Introduction and methods:** Methylglyoxal (MG) is a highly reactive glucose metabolite that is elevated in diabetics and thought to mediate some of the complications associated with diabetes (1) which include gastro-intestinal disturbances. The recent identification of MG as a TRPA1 channel agonist(2) may provide a potential mechanism for this activity. In this study, we have investigated the effect of MG and a TRPA1 agonist, AITC, on contraction and relaxation of distal colon from control rats and diabetic rats (blood glucose >16mmol/L) two weeks after diabetes induction using streptozotocin (STZ, single dose, 65 mg/kg i.p.) treatment. Longitudinal and circular muscle strips of distal colon were removed post mortem from male Wistar rats euthanized with CO<sub>2</sub>/cervical dislocation. The effects of MG and AITC ± TRPA1 antagonist HC-030031 were examined on spontaneous contractions and responses induced by carbachol and electrical field stimulation (EFS) using standard organ bath techniques. Data are mean ± sem, n= 4-6 experiments, statistics are unpaired t-tests.

**Results:** In longitudinal muscle strips a 60 min pre-treatment with 1-10 mM MG resulted in significant increases in the amplitude of spontaneous contractions to levels similar to that seen in diabetic strips; (0.812 ± 0.089 g after MG compared to 0.342 ± 0.04 g control strips and 0.75 g ± 0.108 in STZ strips; p < 0.0001 10mM MG vs control, p<0.001 STZ vs control). 10 mM MG potentiated the contractile response of control longitudinal muscle strips to carbachol and EFS: the maximum response increased from 1.38 g ± 0.214 to 3.27 g ± 0.286 (p<0.0001) and 2.16 g ± 0.323 to 3.64 g ± 0.421 (p<0.05), respectively. 10mM MG still increased EFS induced contractions in the presence of atropine, indicating that MG was acting via a non-cholinergic mechanism. AITC (300µM) also increased EFS responses and in the presence of AITC, 10 mM MG evoked no further increase. Enhancement of EFS responses by MG was blocked with 10 µM HC-030031, a TRPA1 antagonist. 300 µM AITC or 10 mM MG alone induced contraction of longitudinal muscle and this was blocked by HC-030031.

In circular muscle spontaneous activity was increased by pre-treatment with MG 1-10 mM. EFS in the presence of 1 µM atropine induced relaxation in a frequency dependent-manner. MG significantly decreased the maximum relaxation response achieved at 20 Hz from 0.26 g ± 0.036 to 0.055 g ± 0.046 (p<0.05).

**Conclusion:** MG and AITC cause a direct contraction of rat distal colon and enhancement of carbachol and EFS-induced responses. Blockade of these effects with a TRPA1 antagonist indicated that these effects of MG and AITC are mediated by activating the TRPA1 channel via release of excitatory neurotransmitters from enteric neurons. MG may also block inhibitory neurotransmission.

#### References

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