# Bitter Taste Receptors as Regulators of Abdominal Muscles Contraction

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Received February 28, 2019
Accepted July 16, 2019
Epub Ahead of Print October 25, 2019

**Summary**

Bitter taste receptors (TAS2R) are expressed in many non-sensor tissues including skeletal muscles but their function remains unexplored. The aim of this study is to investigate the role of TAS2R in rat abdominal skeletal muscles contractions using denatonium, a TAS2R agonist. Low concentration of denatonium (0.01 mmol/l) caused a significant decrease of amplitudes of the electrical field stimulation (EFS)-induced contractions in abdominal skeletal muscles preparations *in vitro*. This inhibitory effect was significantly reduced when the preparations were pre-incubated with gentamicin (0.02 mmol/l) used as a non-specific inhibitor of $IP_3$ formation or with $BaCl_2$ (0.03 mmol/l) applied to block the inward-rectifier potassium current. All experiments were performed in the presence of pipecuronium in order to block the nerve stimulation of the contractions. The data obtained suggest that denatonium decreases the force of rat abdominal muscles contractions mainly *via* activation of TAS2R, phosphatidylinositol 4,5-biphosphate and its downstream signal metabolites.

**Key words**

Skeletal muscle • Taste receptors • Electric field stimulation • $PIP_2$ • $IP_3$ • Relaxation

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## Introduction

Sweet, umami and bitter taste receptors are widely expressed in non-taste sensory organs. Bitter taste receptors (TAS2R) are involved in the regulation of the physiological processes such as airway smooth muscle cell relaxation and bronchodilation, the secretion of the gastro-intestinal tract, artery relaxation, as well as in the modulation of nervous and immune systems (for review Dalesio *et al.* 2018). These influences are supposed to be related to TAS2R signal pathway which activates G-protein, phospholipase C (PLC), protein kinase C (PKC) and $IP_3$-induced $Ca^{2+}$ release (IICR) in non-taste sensory tissues (Avau *et al.* 2015, Dalesio *et al.* 2018). As a result of this stimulation, cytoplasmic $Ca^{2+}$ concentration increases and several protein targets of PKC change their activities. However, the effects of TAS2R activation in skeletal muscles remain unclear. The aim of this study is to investigate the effect of denatonium, an agonist of TAS2R, on electrical field stimulation (EFS)-induced abdominal skeletal muscles contractions and their dependence on phosphatidyl-inositol 4,5-bisphosphate ($PIP_2$) and its break down. The role of inward-rectifier potassium (Kir) channels on TAS2R-induced effect was also studied.

## Materials and Methods

The experiments were approved by the

Bulgarian Food Safety Agency and the Ethics Committee of the Medical University of Plovdiv, Bulgaria (approval nos. 87/9.01.2014 and 5/29.09.2016 respectively). Male Wistar rat *transversus abdominis muscles* strips were isometrically fixed as previously described (Zagorchev *et al.* 2016). During a period of 20 min the muscle activity was elicited by unipolar EFS used as a control and analysed (Zagorchev *et al.*, 2018). EFS were repeated, square-wave multipulses of supramaximal intensity, 0.5 ms in duration applied at frequency of 5 Hz and 50 Hz for 3 s followed by a 7 s pause. After this, denatonium $10^{-5}$ mol/l, gentamicin $2.10^{-5}$ mol/l, gentamicin $2.10^{-5}$ mol/l and denatonium $10^{-5}$ mol/l, $BaCl_2$ $3.10^{-5}$ mol/l or $BaCl_2$ $3.10^{-5}$ mol/l and denatonium $10^{-5}$ mol/l were added to the organ baths. All experiments were conducted in the presence of $10^{-5}$ mol/l pipecuronium to block the nerve stimulation of the muscles (Youssef *et al.* 1993). For a statistical analysis, SPSS.15 (Chicago, IL, USA) was employed. All data are expressed as mean ± SEM after verifying the normality one-way analysis of variance (ANOVA), Bonferroni Multiple Comparison Test the Paired samples t-test. The number of tested muscle strips is indicated by *n*. Results were considered as statistically significant at $p<0.05$.

## Results

EFS-evoked (5 Hz) single contractions of rat *transversus abdominis muscles* preparations *in vitro* maintain stable but slightly declining amplitudes in the presence of pipecuronium, which was added to block the nerve stimulation of nicotinic acetylcholine receptor (nAChR, time control). Thus 1 min after drug application it was 5.2±0.5 mN, after 5 min - 4.7±0.5 mN and after 15 min - 4.1±0.4 mN (Fig. 1 A, B and C, left column and Fig. 2, left panel, ж). The same event but less pronounced was observed in 50Hz EFS-evoked tetanic contractions (Fig. 1D and Fig. 2, right panel, ж). The addition of denatonium at a concentration of $10^{-5}$ mol/l induced a significant decrease ($p<0.05$, n=12) of the force of single contractions after 5 min to 1.7±0.6 mN and almost complete inhibition after 15 min (0.3±0.1 mN) along with a large decrease ($p<0.05$, n=12) of tetanic contractions after 15 min (2.3±0.5 mN) (Fig. 2A, ●). The inhibition of the contraction force was faster when $5.10^{-5}$ mol/l denatonium was used (data not shown). A low concentration of gentamicin ($2.10^{-5}$ mol/l) was applied into the bath to suppress selectively $IP_3$ generation. This treatment also reduced the force of single and tetanic EFS-induced contractions but with a constant potency during the whole studied time interval (Fig. 1, Fig. 2A, ■). In the presence of simultaneously added $2.10^{-5}$ mol/l gentamicin and $10^{-5}$ mol/l denatonium (Fig. 1, Fig. 2A, ♦) a further decrease in the amplitudes of the contraction force was observed but the effect of denatonium was less pronounced as compared to the single contractions ($p<0.05$, n=12). Thus, the amplitudes of contractions after 5 min were 3.3±0.3 mN, after 10 min - 3.0±0.4 mN, after 15 min - 2.6±0.3 mN and at 20 min - 2.3±0.3 mN. For tetanic contractions, the denatonium suppression of the force of contraction in the presence of gentamicin was weaker: after 10 min it was 5.3±0.5 mN, after 15 min - 5.0±0.5 mN and after 20 min - 4.9±0.4 mN.

Next, we applied $BaCl_2$ to inhibit Kir channels. In the presence of 0.03 mmol/l $Ba^{2+}$ the maximal amplitudes of single contractions slightly declined after 5 min $BaCl_2$ application to 4.3±0.6 mN, to 3.9±0.5 mN after 10 min, to 3.6±0.4 mN after 15 min and to 3.5±0.4 mN after 20 min (Fig. 2B, left panel, ■). In the presence of both 0.03 mmol/l $BaCl_2$ and 0.01 mmol/l denatonium, the amplitudes of contractions were further reduced to 3.6±0.4 mN, 3.3±0.5 mN, 3.0±0.5 mN and to 2.9±0.4 mN after the same time intervals. Similar results of $Ba^{2+}$ or $Ba^{2+}$ and denatonium were obtained on the tetanic contractions.

All these data indicate that low concentrations of gentamicin or $Ba^{2+}$ substantially reduce the inhibitory effect of denatonium on the EFS-induced single and tetanic contractions of abdominal skeletal muscles preparations *in vitro*.

## Discussion

To the best of our knowledge this is the first report on denatonium-dependent regulation of skeletal muscle function. The effective low concentration of denatonium suggests a selective denatonium-TAS2R interaction followed by a G-protein-dependent activation of PLC that breaks down $PIP_2$, into $IP_3$ and diacylglycerol (DAG), as well as weakens the EFS-induced contractions. This hypothesis is supported by the significantly suppressed effect of denatonium in the presence of gentamicin, the latter used to inhibit $IP_3$-formation (Touchberry *et al.* 2014). It is known that application of higher concentrations of gentamicin can decrease the motor neuron stimulation of contraction because it is a competitive inhibitor of nAChR (Amici *et al.* 2005) and thus it reduces the amplitudes of skeletal muscle

contractions. However, under our experimental conditions, neuronal ACh mediation was eliminated and EFS stimulations excited only the skeletal muscles. Additionally, the low concentration of gentamicin greatly increased rather than suppressed the force of contractions in the presence of denatonium. Therefore, we assume that such a side effect is successfully avoided and almost a 'pure' influence of gentamicin on $PIP_2/IP_3/DAG$-signaling is achieved. Also, the effective low concentration of gentamicin suggests that the influence of TAS2R activation on $PIP_2$ metabolism is not completely eliminated and this could be a reason for the partial block of denatonium-induced relaxation.

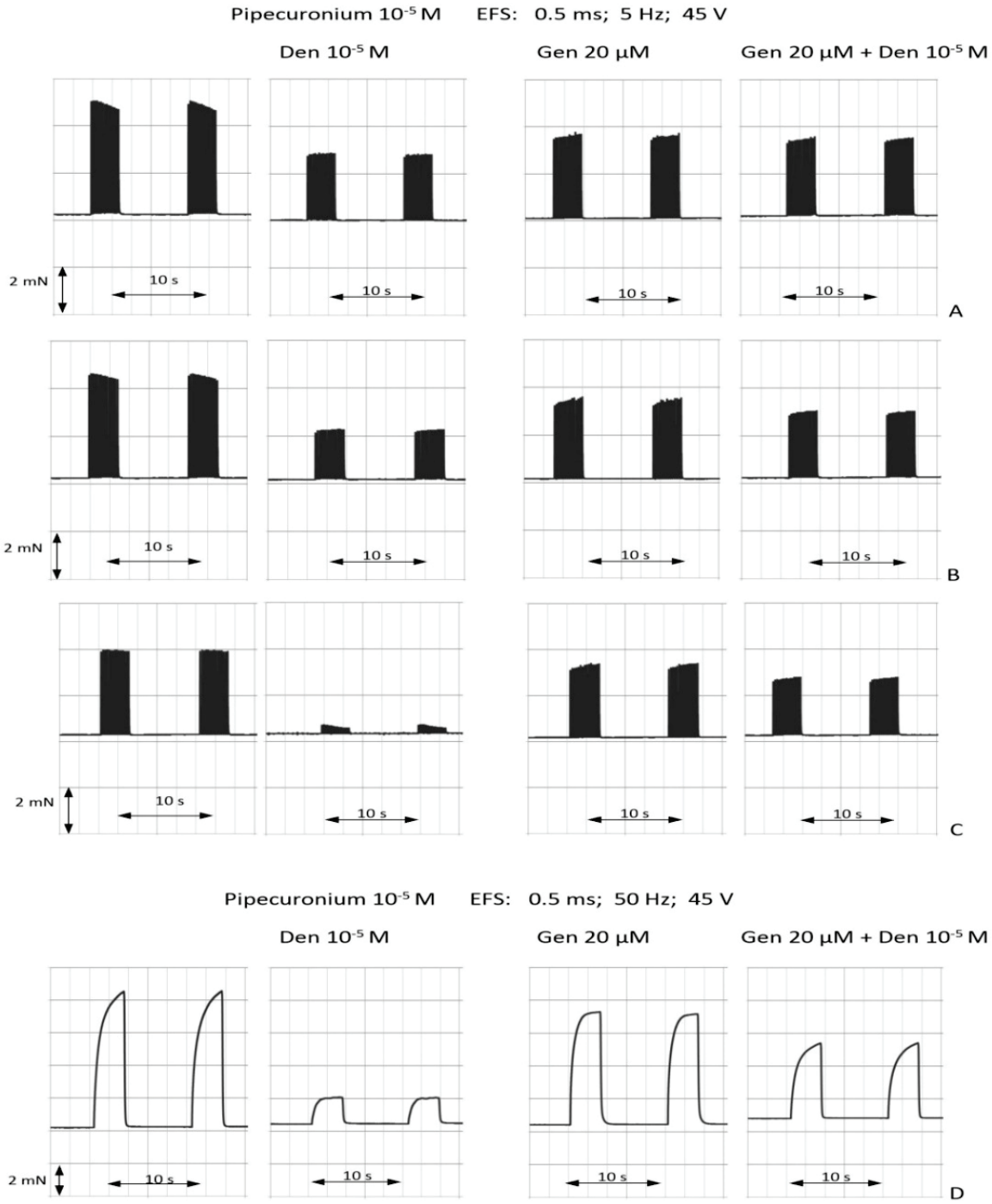


**Fig. 1.** Direct EFS-evoked contractions of *m. transversus abdominis* preparations *in vitro* in the presence of $10^{-5}$ mol/l pipecuronium and after the addition of $10^{-5}$ mol/l denatonium (Den $10^{-5}$ M), of $2.10^{-5}$ mol/l gentamicin (Gen 20 µM) and of $10^{-5}$ mol/l denatonium and $2.10^{-5}$ mol/l gentamicin (Gen 20 µM + Den $10^{-5}$ M). **A)** after 1 min, **B)** after 5 min and **C)** after 15 min, all stimulated with EFS - 0.5 ms, 5Hz, 45V, **D)** 15 min after EFS with duration 0.5 ms, 50Hz, 45V.

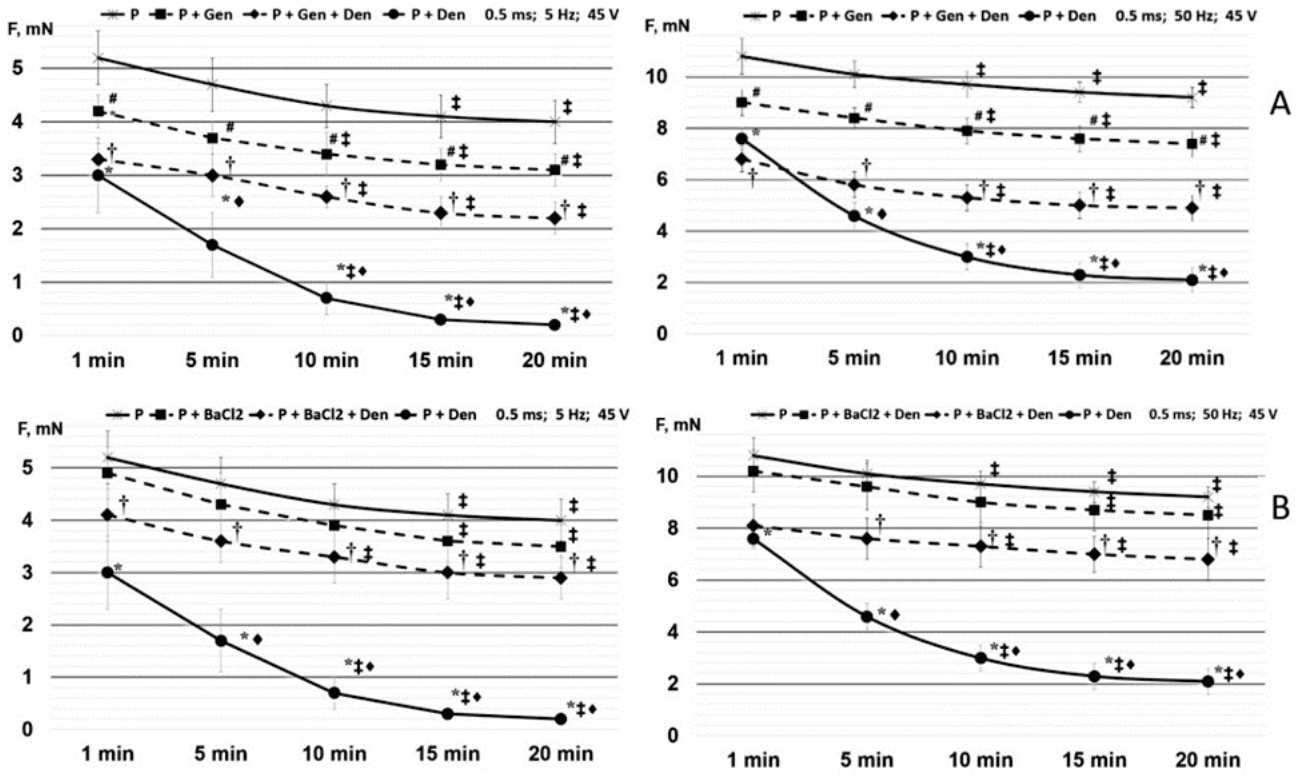


**Fig. 2.** Time dependence of contraction force by direct EFS stimulation: 0.5ms, 5Hz, 45V – left and 0.5 ms, 50Hz, 45V – right. **A)** The curves represent the force in mN in the presence of $10^{-5}$ mol/l pipecuronium (P, ж) for a time control, of $10^{-5}$ mol/l pipecuronium and $2.10^{-5}$ mol/l gentamicin (P + Gen, ■), of $10^{-5}$ mol/l pipecuronium, $2.10^{-5}$ mol/l gentamicin and $10^{-5}$ mol/l denatonium (P + Gen + Den, ♦) and of $10^{-5}$ mol/l pipecuronium and denatonium (P + Den, ●), ‡ p< 0.05, n=12 vs effect on 1 min of the following measurements after 5, 10, 15 и 20 min, **#** p< 0.05 gentamicin *vs* time control, ***** p<0.05, n=12 denatonium *vs* time control, † p< 0.05 n=12 for denatonium *vs* gentamicin and ♦ p<0.05, n=12 for denatonium *vs* denatonium and gentamicin. **B)** The curves represent the force in mN in the presence of $10^{-5}$ mol/l pipecuronium (P, ж) for time control, of $10^{-5}$ mol/l pipecuronium and $3.10^{-5}$ mol/l $BaCl_2$ (P + $BaCl_2$, ■), of $10^{-5}$ mol/l pipecuronium, $3.10^{-5}$ mol/l $BaCl_2$ and $10^{-5}$ mol/l denatonium (P + $BaCl_2$ + Den, ♦) and of $10^{-5}$ mol/l pipecuronium and denatonium (P + Den, ●), ‡ p< 0.05, n=12 vs effect on 1 min of the following measurements after 5, 10, 15 and 20 min, **#** p< 0.05 $BaCl_2$ *vs* time control, ***** p<0.05, n=12 denatonium *vs* time control, † p<0.05, n=12 for denatonium *vs* $BaCl_2$ and ♦ p<0.05, n=12 for denatonium *vs* denatonium and $BaCl_2$.

Another set of experiments targeted Kir channels. It is generally accepted that Kir2.1 and Kir2.2 channels are the prevalent forms of Kir channels in skeletal muscles (DiFranco *et al.* 2015). They are present either on the surface (about 30 %) or in T-tubules (about 70 %) of fibres (DiFranco *et al.* 2015). Kir channels play a crucial role in skeletal muscle excitability by keeping the extracellular concentration of $K^+$ low and thus the resting membrane potential at optimal value (DiFranco *et al.* 2015). Kir2.x channels are very important for keeping the physiological $K^+$ gradients, especially in T-tubules, because of the restricted intra T-tubular space that can be easily saturated by $K^+$ when $K^+$ return (influx) is disturbed. The latter will ultimately lead to membrane depolarization and will complicate the excitation-contraction coupling in the skeletal muscle fibres. Additionally, it is known that Kir2.1 channels has several $PIP_2$ binding sites which directly activate the channels (Soom *et al.* 2001). That is why, we tested Kir channel as possible participants in denatonium induced signaling using low concentration of $BaCl_2$ to block the channel. Similarly to gentamicin, barium ions reduced the effect of denatonium on the amplitudes of the studied contractions. These results suggest a link between Kir channels and TAS2R-induced reduction of amplitudes of single and tetanic contractions of rat abdominal muscles. Our data do not rule out the involvement of other cellular targets of the denatonium signal transduction pathway with significant effect on EFS-induced contractions.

The novel finding of our research is that denatonium regulates the contractility of rat abdominal skeletal muscle via TAS2R, $PIP_2$ and its metabolites. This denatonium effect depends on the availability of Kir channels. Additionally, our study outlines the described preparations as an appropriate object of diverse physiological and pharmacological studies on TAS2R/$PIP_2$/$IP_3$+DAG signaling in skeletal muscles. The functional significance of IICR in them is still not very

clear (Filip *et al.* 2019a, b), although all three $IP_3$ receptor subtypes are expressed there, two of them ubiquitously (Santulli *et al.* 2017).

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

This work was supported by the Bulgarian National Research Fund [grant number: DN 11/15 /18.12. 2017], NATO Science for Peace and Security Programme [grant number: NATO SPS MYP G5266] and NIH R01-DK106964 grant to Georgi V. Petkov.

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