Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Tenore et al., J Nutr Food Sci 2018, 8:1
DOI: 10.4172/2155-9600.1000664

Research Article Open Access

A Crosstalk between Melatonin and Taste-Receptors' Signaling Tunes Quinine-Induced Gut Hormone Secretion in Mice

Gian Carlo Tenore, Sara Bottone, Gennaro Riccio, Nadia Badolati, Mariano Stornaiuolo* and Ettore Novellino

Department of Pharmacy, University of Naples Federico II, Via Montesano 49, 80138 Naples, Italy

Abstract

Quinine consumption has been shown to reduce appetite and food intake in human and mice. Here, tested on two common mouse strains, C_3H/Ibg and C57BL/6J, it exerted a different effect. While quinine reduced weight gain in C_3H/Ibg mice, C57BL/6J were unaffected by the bitter molecule. Among the differences between the two strains, C57BL/6J present a blunted Melatonin production. In this study, we investigate if endogenous Melatonin is playing any role in the different response of C57BL/6J mice to quinine. The effect of dietary supplementation with Melatonin as well as of endogenous gastrointestinal and pineal produced Melatonin was investigated by supplementing quinine diet with pure Melatonin, L-Tryptophan or by reversion of light/dark cycle, respectively. The consumption of Melatonin reverts the phenotype and makes C57BL/6J mice sensitive to quinine. Similarly, quinine potency in C_3H/Ibg mice augments upon supplementation with exogenous Melatonin or upon increase of Melatonin endogenous levels. *In vivo,* as well as in *in vitro* cell cultures, Melatonin Receptor modulation inhibits quinine dependent secretion of Ghrelin, while potentiates quinine dependent secretion of Cholecystokinin. Acting *via* Melatonin Receptors, Melatonin tunes the effect of quinine, reducing and potentiating its effect in enteroendocrine cells of the upper and the lower digestive tract, respectively. Our results indicate that signaling pathways activated by Melatonin tunes the activity of Bitter Receptors located in the gastrointestinal tract.

Keywords: Melatonin; Quinine; Melatonin receptor; Bitter taste receptor; Weight gain; Gut hormones

Introduction

Quinine is a bitter tasting molecule extracted from the bark of the cinchona tree and commonly used as food additive to flavor tonic water and bitter lemon.

Like most of the bitter tastants, quinine binds to the members of the Bitter Taste Receptor Family (TAS2Rs), a family of GPCR expressed in taste receptor cells (TRCs) of the oral cavity [1]. TAS2Rs recognize bitter molecules (mostly alkaloids) and warn from the ingestion of these potentially toxic exogenous substances [2]. More TAS2Rs coexist in the same TRC and trigger a unique intracellular response independently from the nature of the bitter tastant [3-5]. Ligand binding activates heterotrimeric G proteins (Gustducin or Transducin), stimulates Phospholipase C β2 (PLC-β2) and synthesis of Inositol-3-phosphate (IP3), ultimately resulting in the release of Ca^{2+} from internal stores and secretion of neurotransmitters. Some TAS2Rs are broadly tuned and bind to a wide range of structurally distinct compounds, while others are more narrowly tuned. Quinine has been shown to bind and stimulate the activity of 9 members of the TAS2Rs'family.

TAS2Rs are not only expressed in TRCs but also in lungs, brain and testis [6-12]. Recently, several TAS2Rs, including those binding to quinine, have been shown to be expressed in the enteroendocrine cells (EECs) of the Gastrointestinal (GI) Tract [13-16]. In EECs, TAS2Rs stimulation by bitter tastants induces secretion of gut hormones like Ghrelin, Cholecystokinin and affects gastric mobility [17-20].

It has been widely demonstrated that consumption of quinine affects appetite in human and rodents and affect body weight gain in mouse and rats [21,22]. Quinine effect on food intake goes beyond palatability or aversion to the bitterness of the molecule and mostly relies on the signaling elicited upon quinine binding to TAS2Rs localized in EECs [20]. In virtue of the peculiar ability of quinine to induce weight loss, extracts from cinchona tree can be allocated among those nutraceuticals endowed with anti-obesity potential.

In order to identify the quinine dosage necessary to achieve maximal reduction of weight gain, we planned a dose-response analysis of the effect of quinine on two common mouse strains, namely C_3H/Ibg and C57BL/6J. In our study, the two mouse strains responded differently to quinine consumption. Quinine supplemented food affected weight gain of C_3H/Ibg but not of C57BL/6J mice.

We here show that one the reason behind the different response of C57BL/6J mice is their inability to produce Melatonin and prove, to the best of our knowledge for the first time, the existence of an interplay between TAS2Rs' and Melatonin signaling.

Materials and Methods

Reagents

Chemicals and reagents used were HPLC grade. Melatonin and L-Tryptophan were from FARMALABOR (Italy). Quinine-HCl and Luzindole were from AlfaAesar (U.K). Mother stock solutions of the compounds were dissolved in absolute ethanol or water.

Animals

All experiments were performed according to Italian legislation for the protection of animals. C57BL6/J and C_3H/Ibg mice were purchased from Charles River (Germany). All experiments were performed with

***Corresponding author:** Mariano Stornaiuolo, PhD, Department of Pharmacy, University of Naples Federico II, Via Montesano 49, 80138 Naples, Italy, Tel: +39 081 670117; E-mail: mariano.stornaiuolo@unina.it

Received January 02, 2018; **Accepted** January 09, 2018; **Published** January 16, 2018

Citation: Tenore GC, Bottone S, Riccio G, Badolati N, Stornaiuolo M, et al. (2018) A Crosstalk between Melatonin and Taste-Receptors' Signaling Tunes Quinine-Induced Gut Hormone Secretion in Mice. J Nutr Food Sci 8: 664. doi: 10.4172/2155-9600.1000664

3.3 months old mice. Twenty-four groups of 8 mice each were studied. Animals were housed under conventional conditions with access to food and water as described in the text. For study 1, mice were fed a AIN 93 G diet supplemented or not with 0.1% or 0.01% quinine HCl for 80 days. For study 2, beverages were supplemented with Melatonin [2.4mg/Kg/die]. For study 4 beverages were supplemented with L-Tryptophan [0.4g/Kg/die]. Body weight and body composition were measured throughout the study. No blinding was done and no randomization was used. For study 5, overnight-fasted C_3H/lbg mice received an intra-gastrically gavage of either 200 µL of sterile H_2O or of quinine HCl (0.1% w/v). At the indicated time points, mice were anesthetized with 3% isoflorane and blood was collected from the abdominal aorta for measurement of Ghrelin and Cholecystokinin levels. C_3H/lbg mice from study 1-3 were used for study 5 as described in the text.

Cell cultures

CaCo-2, and AGS cells were grown in DMEM (#41965-039, GIBCO, Thermo Fisher Scientific) supplemented with 10% FBS (#10270, GIBCO), Glutamax (#35050-061, GIBCO) and Pen/Strep (#15070-063, GIBCO). When indicated media were replaced with serum-free medium containing Melatonin (1µM), quinine (1 mM) and/or Luzindole (10 µM) or a combination of them. After 6 hours of treatment 200 µl of tissue supernatants were transferred to ELISA NUNC Maxisorb plates for 16 hours at 4°C and processed to measure Ghrelin and Cholecystokinin levels.

ELISA: Ghrelin and Cholecystokinin measurements in tissue supernatant and blood samples were performed using a Human Ghrelin ELISA KIT (Millipore) and a Human CCK ELISA KIT (Sigma), respectively. Anti-Ghrelin antibody (SantaCruz (California) was used at the dilution of 1:250 and anti-Cholecystokinin (SantaCruz (California) was used at the dilution of 1:250.

Statistical analysis

Results are presented as means ± S.E.M. Data were analyzed with ANOVA test followed by a Tukey-Kramer post hoc test for the estimation of stochastic probability in intergroup comparisons (PRISM 6.0). Significance was accepted at the 5% level.

Results

Quinine affects weight gain and fat mass in C_3H/Ibg but not in C57BL/6J mice (study 1)

Male C57BL/6J and C_3H/Ibg mice were fed quinine supplemented pellets for 80 days. Quinine percentage in the food was 0.01% w/w (which is the highest concentration allowed in drinks for human consumption) or 0.1% w/w [21,22]. 0.01% quinine had no effect on the body weight, independently from the mouse strain tested (Figure 1A-1B). On the contrary, at the dosage of 0.1% w/w, quinine affected body weight gain of C_3H/Ibg but not of C57BL/6J mice (Figure 1A-1B). Compared with C_3H/Ibg mice consuming a regular diet, C_3H/Ibg mice consuming 0.1% quinine supplemented diet gained less body weight (5.20 ± 0.30 g vs. 3.40 ± 0.21 g; $p<0.05$, Figure 1A) and less fat mass (5.10 ± 0.20 g vs. 2.10 ± 0.17 g; $p<0.05$, Figure 1C). On the contrary, there was no significant difference in lean mass between C_3H/Ibg mice fed a normal or a 0.1% quinine diet (Figure 1D). No significant differences could be measured in body weight, fat mass or lean mass (data not shown) between C57BL/6J mice consuming regular diet and those consuming quinine supplemented diet (Figure 1B).

The absence of Melatonin contributes to explain the different response of C57BL/6J mice to quinine (study 2)

Despite C_3H/Ibg and C57BL/6J derive from the same inbred C57BL/6 strain, they differ on many phenotypes [23,24]. Filtering their differences using keywords linked to food consumption, it came out that one of these differences is that C_3H/Ibg strain produces a proficient level of Melatonin, while the second presents a blunted Melatonin production. This arises as consequence of a mutation occurring in C57BL/6J mice and abolishing the expression of Arylalkylamine N-acetyltransferase a key enzyme in the Melatonin synthesis [25]. Since Melatonin has been already involved in energy expenditure and body weight regulation, we thought it could have been interesting to verify if Melatonin was contributing to the different response of the two strains [26-28].

To demonstrate that the different responses of the two strains could partially depend on the absence of endogenous Melatonin, beverages of C57BL/6J mice were supplemented with Melatonin (final concentration of 2.5mg/kg/day). In the presence of Melatonin, C57BL/6J mice consuming 0.1% quinine supplemented diet for 80 days had lower body weight than C57BL/6J fed a 0.1% quinine diet and receiving unsupplemented beverages (3.15 ± 0.40 g vs. 5.00 ± 0.38 g; $p<0.05$, (Figure 2A)).

Interestingly, in the presence of Melatonin, C57BL/6J mice consuming 0.01% quinine supplemented diet gained less weight compared to those receiving 0.01% quinine andt unsupplemented beverages (2.89 ± 0.30 g vs. 5.70 ± 0.38 g $p<0.05$, Figure 2A). The presence of Melatonin in the beverages positively influenced C_3H/Ibg mice responses to quinine as well. In the presence of the Melatonin, C_3H/Ibg mice consuming 0.01% quinine supplemented diet had lower body weight than those fed a 0.01% quinine diet and unsupplemented beverages (4.00 ± 0.28 vs. 5.00 ± 0.18, $p<0.001$, Figure 2A-2B). Our data thus suggest that Melatonin reverts the phenotype of C57BL/6J mice and, independently from the strain, it potentiates the effect of a low dosage quinine diet.

Endogenous pineal Melatonin influences responses to quinine (study 3)

Similarly to human, endogenous Melatonin production in laboratory mice, like C_3H/Ibg, is triggered by darkness (pineal gland production) [29]. More precisely, Melatonin levels in C_3H/Ibg have been finely analyzed and shown to peak at two hours before dawn. To monitor the contribution of pineal gland produced endogenous Melatonin on quinine activity, C_3H/Ibg mice were maintained on either a normal 12 h light/12 h dark cycle (lights on 09:00 a.m. to 09:00 p.m.) or a reverse 12 h dark/12 h light cycle (lights on 09:00 p.m. to 09:00 a.m.). Both groups were fed at 06:00 p.m. and allowed to access food for 12 hours. Quinine affected body weight of mice maintained in the reversed light/dark cycle more than those maintained at normal light/dark cycle. C_3H/Ibg mice maintained in the reversed light/dark cycle and consuming 0.01% quinine supplemented diet gained less body weight (4.50 ± 0.30 g vs. 5.10 ± 0.20 g $p<0.05$, Figure 3A) than mice kept at normal light/cycle. There was no significant difference in body weight of C_3H/Ibg fed a normal diet and maintained under normal or reversed light/dark cycle. Similarly, we did not register significant differences in body weight of C57BL/6J mice (either in the presence or in the absence of quinine) maintained under normal or reversed light/dark cycle (data not shown).

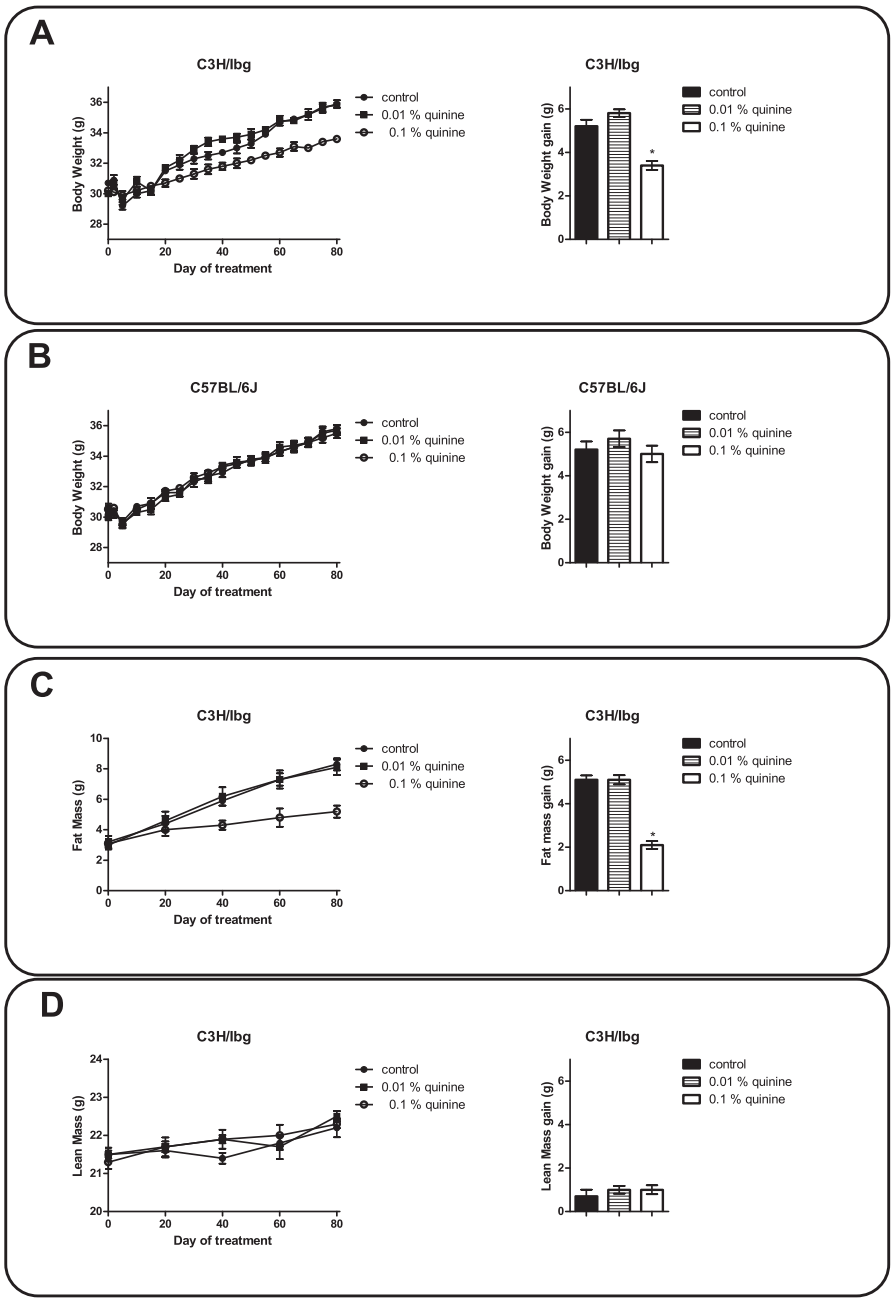

Figure 1: Effect of quinine on body weight gain in mice. Body weight increase (expressed in grams) of C3H/Ibg (A) and C57BL/6J (B) mice receiving for 80 days pellets supplemented with the indicated amount of quinine (expressed as % w/w of total food). Fat mass (C) and lean mass (D) of C3H/Ibg mice receiving pellets supplemented or not with the indicated amount of quinine. (Values are reported as mean ± S.E.M, n=8 (*P<0.05)).

Endogenous GI Melatonin influences responses to quinine (study 4)

Melatonin is synthesized in EECs throughout the gut, and these cells have been reported to be the major source of L-Tryptophan (L-Trp) induced increase of circulating Melatonin [30,31]. Production is controlled by feeding and is increased by meal enriched in L-Trp, the Melatonin precursor. Oral administration of L-Trp caused a rapid and dose-dependent elevation of circulating Melatonin in murine models [32]. In the GI, Melatonin acts as paracrine gut hormone even if the consequence of its activity is far from being clear and fully elucidated [29]. To monitor the contribution of GI produced endogenous Melatonin on quinine effect, beverages of C_3H/Ibg mice were supplemented with L-Trp (0.4 g/kg/die). In the presence of L-Trp, quinine affected mice body weight more than those receiving unsupplemented water as beverage. As seen in study 3, in the presence of L-Trp, the 0.01% quinine diet exerted an appreciable effect on body weight. C_3H/Ibg mice fed a 0.01% quinine diet and L-Trp gained less weight (4.30 ± 0.58 g vs. 5.00 ± 0.18 g $p<0.05$, Figure 3B) than those fed a 0.01% quinine diet and receiving unsupplemented beverages. L-Trp

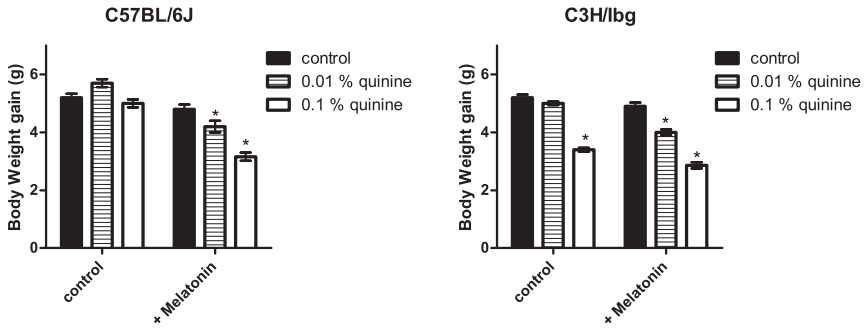

Figure 2: Melatonin reverts the phenotype of C57BL/6J mice. Body weight increase (expressed in grams) of C3H/Ibg (A) and C57BL/6J (B) mice receiving for 80 days pellets supplemented with the indicated amount of quinine (expressed as % w/w of total food). When indicated mice were watered with Melatonin containing beverages (2.5mg/kg/day). (Values are reported as mean ± S.E.M, *n*=8 (*P<0.05)).

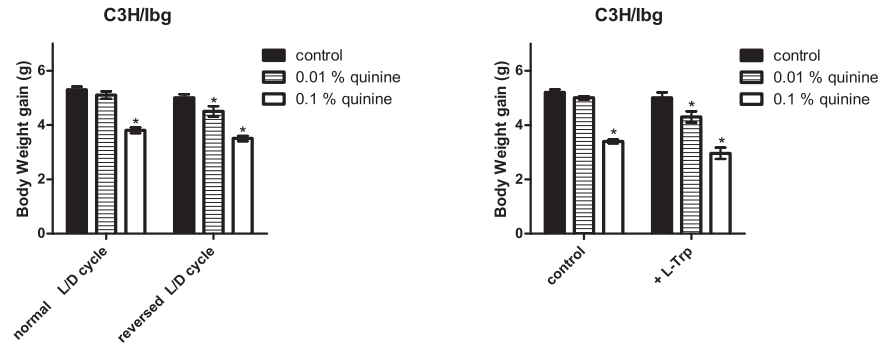

Figure 3: Endogenously produced Melatonin potentiates quinine effect on C57BL/6J mice weight increase. Body weight increase (expressed in grams) of C3H/Ibg mice receiving for 80 days pellets supplemented with the indicated amount of quinine (expressed as % w/w of total food). When indicated beverages were supplemented with L-Trp (0.4mg/kg/day). When indicated mice were kept in a reverse light dark cycle. (Values are reported as mean ± S.E.M, *n*=8 (*P<0.05)).

did not affect body weight of C_3H/Ibg maintained under normal diet. Similarly, we did not register significant differences in body weight of C57BL/6J mice (both in the presence and in the absence of quinine) maintained under unsupplemented or L-Trp supplemented beverages (data not shown).

Melatonin tunes bitter-induced gut hormone secretion

To monitor the effect of quinine and Melatonin on gut hormones levels in blood, CH3/lbg mice received an intragastric gavage of a solution of 0.1% quinine supplemented or not with Melatonin. Compared to gavages of pure water, mice receiving 0.1% quinine presented increased level of both Ghrelin (1h upon gavage, 1480 ± 90 pg/ml vs. 1990 ± 130 pg/ml; $p<0.05$, Figure 4A) as well as in CCK blood levels (2h upon gavage, 200 ± 60 pg/ml vs. 820 ± 60 pg/ml; $p<0.05$,Figure 4B), as already shown [17]. Mice receiving a solution containing only Melatonin showed no significant differences in Ghrelin and CCK blood level compared to control mice (medians of 1520 ± 190 pg/ml and 330 ± 60 pg/ml for Ghrelin and CCK, respectively). Surprisingly, intragastric gavage of a solution 0.1% quinine supplemented with Melatonin resulted in Ghrelin and CCK blood levels diminished and increased, respectively (medians of 1480 ± 160 pg/ml and 1130 ± 60 pg/ml for Ghrelin and CCK, respectively).

These results seem to indicate that, at least in C_3H/lbg mice, Melatonin potentiates quinine dependent CCK secretion, while reduces quinine dependent Ghrelin secretion.

Melatonin receptor influences TAS2R receptor activity in cultured cells

Melatonin signaling acts *via* MNTR1A and MNTR1B, two Melatonin Receptor localized on the Plasma Membrane of the cells [33,29]. Melatonin binding to MNTRs has been shown to reduce cAMP levels by activation of inhibitory heterotrimeric Go/i proteins as well to stimulate Inositol-3-phosphate (IP3) by activation of Gq proteins. The outcome of Melatonin stimulation is often cell dependent, with cells particularly enriched in MNTR1A primarily linked to inhibitory signaling while MNTR1B expressing cells activating the Gq pathway [34-36].

We thought that a possible biochemical explanation for Melatonin and quinine interplay could have been a crosstalk between their cognate receptors MNTRs and TAS2Rs. To test this hypothesis we moved to *in vitro* models of GI cells.

Human gastric adenocarcinomas AGS cells express TAS2Rs and have been shown to respond to stimulation by secreting Ghrelin [37]. Moreover, they express MNTRs and thus represents a suitable *in vitro* biological system for our analysis [38,39]. As expected, treatment with quinine induced Ghrelin secretion in AGS cells (Figure 4C). However, Ghrelin secretion was inhibited when quinine was supplemented together with Melatonin. To prove Melatonin effect on quinine activity being dependent on MNTRs' activity, we pre-incubated AGS cells with Luzindole, a Melatonin Receptor inhibitor. In the presence of

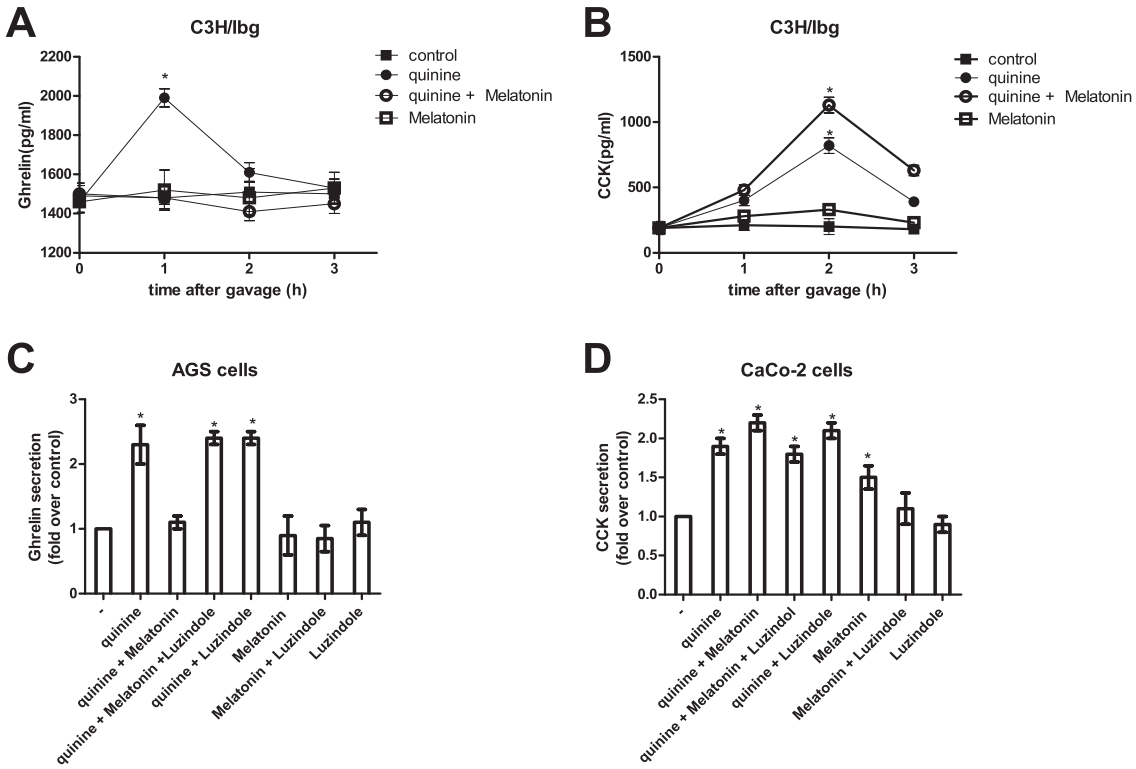

Figure 4: Melatonin tunes quinine dependent gut hormones secretion *in vivo* and *in vitro*. (A-B). Ghrelin (A) and of CCK (B) blood levels in C3H/lbg mice receiving intragastric gavages of 0.01% quinine and/or 1 μM Melatonin. (C-D) Increase in the amount of gut hormones secreted by AGS (C) and CaCo-2 (D) cells upon stimulation with 1 mM quinine, 1 μM Melatonin, 10 μM Luzindole or combinations of them. (Values indicate the fold increase in gut hormone secretion compared to untreated cells (-). Fold increase are reported as mean ± S.E.M., n=3 (*P<0.05)).

Luzindole, the inhibitory effect of Melatonin was abolished, confirming the involvement of MNTRs [40].

As *in vitro* model of CCK secreting cells, we made use of colon-rectal cancer $CaCo_{-2}$ cells. These cells express TAS2Rs and have been shown to respond to bitter taste receptor stimulation by secreting CCK [20]. Treatment with quinine was able to induce CCK secretion in $CaCo_{-2}$ cells (Figure 4C). When quinine was supplemented together with Melatonin, the amount of secreted CCK was higher than the one obtained upon treatment with quinine alone. As shown AGS cells, also in $CaCo_{-2}$ cells Melatonin effect was abolished by Luzindole confirming the involvement of MNTRs' activity.

Quinine and Melatonin tune short/term food intake (study 5)

After 80 days of consuming a diet supplemented with 0.1% quinine, in the presence or in the absence of Melatonin or L-Tryptophan in the beverage, C_3H/Ibg mice were switched to unsupplemented diet for 4 hours. During that period, we measured the amount of food consumed per hour to estimate appetite of the mice. Compared to control mice, 0.1% quinine mice manifested a loss of appetite and consumed less food (food intake per hour of 0.8 ± 0.2 vs. 1.2 ± 0.1, p<0.05) than control mice. A similar reduction in food intake could be registered for mice fed a 0.01% quinine diet in the presence of beverages containing Melatonin or L-Trp (Figure 5).

However, on a longer period, C_3H/Ibg mice regained appetite once they were switched to a normal diet. In 4 weeks, the weight increase was similar between control mice and those fed a 0.1% quinine diet, in

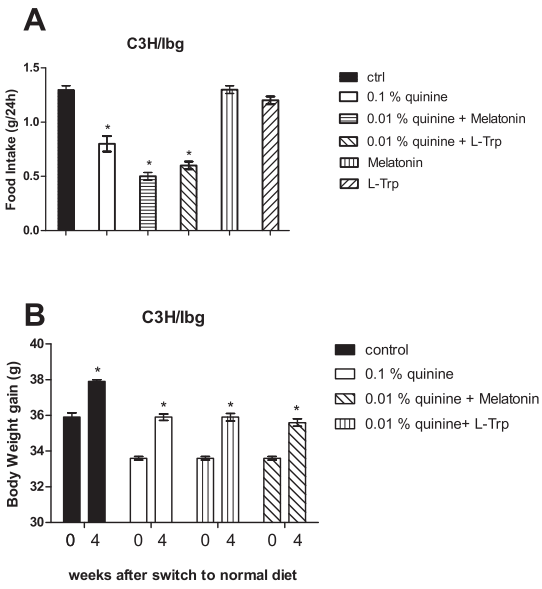

Figure 5: Melatonin/quinine synergism tunes short/term food intake. (A) Food consumption (expressed in grams) of C3H/lbg mice fed the indicated diets for 80 days and, then, kept for 4 hours in the presence of unsupplemented food (Values are reported as mean ± S.E.M, n=8. (*P<0.05)). (B) Body weight increase (expressed in grams) of C3H/lbg mice fed the indicated diet for 80 days to then receive for 4 weeks unsupplemented food (Values are reported as mean ± S.E.M, n=8 (*P<0.05)).

the presence or in the absence of Melatonin. These data all suggest that supplementation of quinine does not affect food intake permanently.

Discussion and Conclusion

Sensory receptors localized on taste buds couple toxic compounds binding to a bitter sensation. This represents, undoubtedly, a successful strategy to avoid noxious food ingestion. While i) TAS2Rs localization in EECs and ability to accelerate ii) gastric emptying and iii) secretion of satiety hormones can all be explained as an additional level of defense against intoxication, the real function of bitter receptors in the GI is not yet complete. It is nowadays widely accepted that is necessary to attribute more than a simple sensory activity to TAS2Rs and start reconsidering their function in the context of non-gustatory environments [12,18,19].

Consumption of the bitter molecule quinine has been shown to reduce appetite and food intake in humans. In virtue of this activity, extracts from cinchona tree can be allocated among those supplements endowed with anti-obesity potential. Here, while trying to identify a plausible explanation for the different response to quinine of two mouse strains, we ended up proving that Melatonin and quinine have a synergistic effect on weight gain reduction in mice. According to our data, this is due to an *in vivo* interplay between Melatonin and TAS2Rs. Considering the plethora of different signaling pathways controlling the activity of receptors localized on the EECs and orchestrating gut hormone secretion in the G.I., an analysis of the signaling network linked to TAS2Rs will definitely help to clarify the scenario in which these receptors act. Upon binding to MNTRs, Melatonin potentiates quinine dependent release of the satiety hormone CCK, while inhibits quinine dependent release of the appetite hormone Ghrelin. The different outcome of the synergism between Melatonin and Quinine is intriguing and definitely worthy of further investigation. It could be possible to envisage, however, that the different G proteins involved in the signaling elicited by MNTRs might play a role in the different influence that Melatonin/quinine synergism may exert on CCK and Ghrelin secreting EECs, respectively.

The overall outcome of our research is that Melatonin potentiates quinine effect on weight gain reduction in mice. Melatonin has been already involved in energy expenditure and body weight regulation [28]. Melatonin inhibits release of insulin from pancreatic β-cells [41]. Pinealectomy in rats causes body weight gain while, on the contrary, selective agonists of MTNRs like piromelatine (NEU-P11) and Ramelteon, body weight loss [42]. Our data suggest that the consumption, in combination, of alimentary doses of quinine (0.01% w/w, which is the concentration allowed in drinks for human consumption) and Melatonin could represent a useful dietary supplement to control compulsive hunger and weight gain in people affected by obesity, nocturnal food craving, and other food-related diseases.

Acknowledgments

This work was financed by AIRC, Associazione Italiana Ricerca sul Cancro (MFAG 17651 to M.S.).

Author Contributions

S.B., G.R. and N.B. conducted all the biological experiments. M.S., E.N. and G.C.T. designed the experiments and wrote the paper.

References

1. Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS (2006) The receptors and cells for mammalian taste. Nature 444: 288-294.
2. Roper SD (2013) Taste buds as peripheral chemosensory processors. Semin Cell Dev Biol 24: 71-79.
3. Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, et al. (2000) A novel family of mammalian taste receptors. Cell 100: 693-702.
4. Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS (2005) The receptors and coding logic for bitter taste. Nature 434: 225-229.
5. Spector AC, Kopka SL (2002) Rats fail to discriminate quinine from denatonium: Implications for the neural coding of bitter-tasting compounds. J Neurosci 22: 1937-1941.
6. Caicedo A, Pereira E, Margolskee RF, Roper SD (2003) Role of the G-protein subunit alpha- gustducin in taste cell responses to bitter stimuli. J Neurosci 23: 9947-9952.
7. Zhang Y, Hoon MA, Chandrashekar J (2003) Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways. Cell 112: 293-301.
8. Meyerhof W, Batram C, Kuhn C (2010) The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors. Chem Senses 35: 157-170.
9. Deshpande DA, Wang WCH, McIlmoyle EL (2010) Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nat Med 16: 1299-1304.
10. Singh N, Vrontakis M, Parkinson F, Chelikani (2011) Functional bitter taste receptors are expressed in brain cells. Biochem Biophys Res Commun 406: 146-151.
11. Xu J, Cao J, Iguchi N, Riethmacher D, Huang L (2013) Functional characterization of bitter-taste receptors expressed in mammalian testis. Mol Hum Reprod 19: 17-28.
12. Finger TE, Kinnamon SC (2011) Taste isn't just for taste buds anymore. F1000 Biol Rep 3: 1-7.
13. Wu SV, Rozengurt N, Yang M, Young SH, Sinnett-Smith J (2002) Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. Proc Natl Acad Sci USA 99: 2392-2397.
14. Latorre R, Huynh J, Mazzoni M (2016) Expression of the Bitter taste receptor, T2R38, in enteroendocrine cells of the colonic mucosa of overweight/obese vs. Lean subjects. PLoS One 11.
15. Prandi S, Bromke M, Hübner S (2013) A subset of mouse colonic goblet cells expresses the bitter taste receptor Tas2r131. PLoS One 8.
16. Rozengurt N, Wu SV, Chen MC, Huang C, Sternini C, et al. (2006) Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon. Am J Physiol Gastrointest Liver Physiol 291: G792-802.
17. Janssen S, Laermans J, Verhulst PJ, Thijs T, Tack J, et al. (2011) Bitter taste receptors and α- gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. Proc Natl Acad Sci 108: 2094-2099.
18. Depoortere I (2014) Taste receptors of the gut: Emerging roles in health and disease. Gut 63: 179-190.
19. Behrens M, Meyerhof W (2011) Oral and extraoral bitter taste receptors. Results Probl Cell Differ 52: 87-99.
20. Jeon TI, Seo YK, Osborne T (2011) Gut bitter taste receptor signalling induces ABCB1 through a mechanism involving CCK. Biochem J 438: 33-37.
21. Cettour-Rose P, Bezençon C, Darimont C, le Coutre J, Damak S (2013) Quinine controls body weight gain without affecting food intake in male C57BL6 mice. BMC Physiol 13: 5.
22. Heybach JP, Boyle PC (1982) Dietary quinine reduces body weight and food intake independent of aversive taste. Physiol Behav 29: 1171-1173.
23. Mekada K, Abe K, Murakami A (2009) Genetic Differences among C57BL/6 Substrains. Exp Anim 58: 141-149.
24. Bryant CD (2011) The blessings and curses of C57BL/6 substrains in mouse genetic studies. Ann N Y Acad Sci 1245: 31-33.
25. Roseboom PH, Namboodiri MA, Zimonjic DB (1998) Natural melatonin "knockdown" in C57BL/6J mice: Rare mechanism truncates serotonin N-acetyltransferase. Mol Brain Res 63: 189-197.
26. Larcher S, Benhamou PY, Pépin JL, Borel AL (2015) Sleep habits and diabetes. Diabetes Metab 41: 263-271.

27. Maury E, Hong HK, Bass J (2014) Circadian disruption in the pathogenesis of metabolic syndrome. Diabetes Metab 40: 338-346.

28. Jenwitheesuk A, Nopparat C, Mukda S, Wongchitrat P, Govitrapong P (2014) Melatonin regulates aging and neurodegeneration through energy metabolism, epigenetics, autophagy and circadian rhythm pathways. Int J Mol Sci 15: 16848-16884.

29. Kennaway DJ, Voultsios A, Varcoe TJ, Moyer RW (2002) Melatonin in mice: Rhythms, response to light, adrenergic stimulation, and metabolism. Am J Physiol Regul Integr Comp Physiol 282: R358-65.

30. Chen CQ, Fichna J, Bashashati M, Li YY, Storr M (2011) Distribution, function and physiological role of melatonin in the lower gut. World J Gastroenterol 17: 3888-3898.

31. Bubenik GA (2002) Gastrointestinal melatonin: Localization, function, and clinical relevance. Dig Dis Sci 47: 2336-2348.

32. Huether G, Poeggeler B, Reimer A, George A (1992) Effect of tryptophan administration on circulating melatonin levels in chicks and rats: Evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci 51: 945-953.

33. Boutin JA, Marcheteau E, Hennig P (2008) MT3/QR2 melatonin binding site does not use melatonin as a substrate or a co-substrate. J Pineal Res 45: 524-531.

34. Lopez-Gonzalez MA, Guerrero JM, Rojas F, Delgado F (2000) Ototoxicity caused by cisplatin is ameliorated by melatonin and other antioxidants. J Pineal Res 28: 73-80.

35. Negi G, Kumar A, Sharma SS (2011) Melatonin modulates neuroinflammation and oxidative stress in experimental diabetic neuropathy: Effects on NF-kB and Nrf2 cascades. J Pineal Res 50: 124-131.

36. Sugden D (1993) Melatonin receptors. Receptor 1993: 1-2.

37. Pazos Y, Alvarez CJ, Camina JP, Casanueva FF (2007) Lysophosphatidic acid inhibits ghrelin secretion in the human gastric adenocarcinoma AGS cell line: Role of mitogenic activated protein kinase signaling pathway. Febs J 274: 5714-5726.

38. Li W, Fan M, Chen Y (2015) Melatonin induces cell apoptosis in AGS cells through the activation of JNK and P38 MAPK and the suppression of nuclear Factor-Kappa B: A novel therapeutic implication for gastric cancer. Cell Physiol Biochem 37: 2323-2338.

39. Xin Z, Jiang S, Jiang P (2015) Melatonin as a treatment for gastrointestinal cancer: A review. J Pineal Res 58: 375-387.

40. Zlotos DP, Jockers R, Cecon E, Rivara S, Witt-Enderby PA (2014) MT1 and MT2 melatonin receptors: Ligands, models, oligomers, and therapeutic potential. J Med Chem 57: 3161-3185.

41. Wolden-Hanson T, Mitton DR, McCants RL (2000) Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology 141: 487-497.

42. Puchalski SS, Green JN, Rasmussen DD (2003) Melatonin effect on rat body weight regulation in response to high-fat diet at middle age. Endocrine 21: 163-168.