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# Investigating Metabolic Gender Differences with Melanocortin Antagonist SKY 2-23-7

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## Introduction

The melanocortin system has been implicated in various physiological functions including pigmentation [1,2], sexual function [3], cardiovascular function [4], memory [5], and energy homeostasis [6-10]. The energy homeostasis functions have been attributed to the melanocortin 3 (MC3R) and melanocortin 4 receptors (MC4R) [6-10]. The intracerebroventricular (ICV) administration of melanocortin agonists has been reported to significantly decrease food intake, whereas administration of antagonists is reported to significantly increase food intake [9,10]. Therefore, melanocortin agonist ligands could serve as a potential treatment for obesity, and melanocortin antagonist ligands could serve as a treatment for cachexia. However, some limitations of the melanocortin ligands do exist such as modulating sexual function [3] and blood pressure [4]. It is, therefore, of interest to develop melanocortin ligands with unique pharmacologies which are void of these undesired effects.

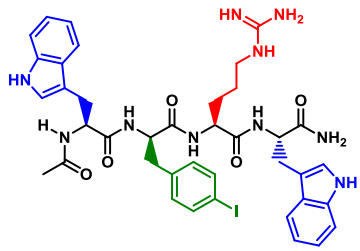


*Fig. 1. Structure SKY2-23-7 Ac-Trp-(pI)DPhe-Arg-Trp-NH2.*

SKY 2-23-7 is a tetrapeptide which was identified in our laboratory with the sequence Ac-Trp-(p-I)DPhe-Arg-Trp-$NH_2$ (Figure 1) [11]. It was discovered through a double substitution strategy of the melanocortin core His-Phe-Arg-Trp sequence. SKY 2-23-7 was characterized as a weak antagonist ($pA_2$=5.43±0.16) at the mMC3R and a strong antagonist ($pA_2$=7.83±0.16) at the mMC4R (Figure 2). Distinctly there was minimal mMC3R agonist activity up to 100 µM which is not commonly observed for a mMC3R/mMC4R antagonist such as SHU9119 [11]. The current study investigated the *in vivo* effects of this unique pharmacology *via* intracerebroventricular (ICV) administration of SKY 2-23-7 in male and female mice which gave evidence of sex specific differences.

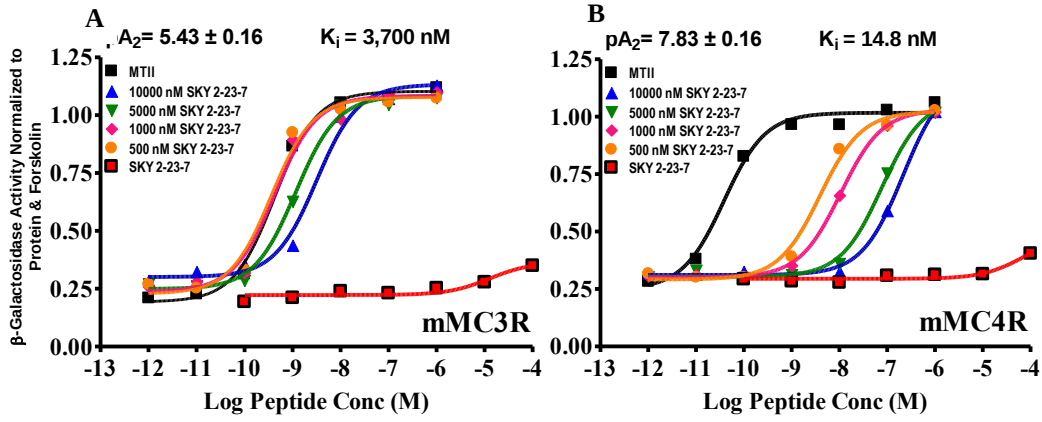


*Fig. 2. Reported in vitro pharmacology of SKY2-23-7 at the mMC3R and mMC4R [11]. The $pA_2$ values were found by a Schild analysis [12]. The $K_i$ was calculated by the equation $pA_2$=-log($K_i$). Figure modified from Doering, et al. 2015.*

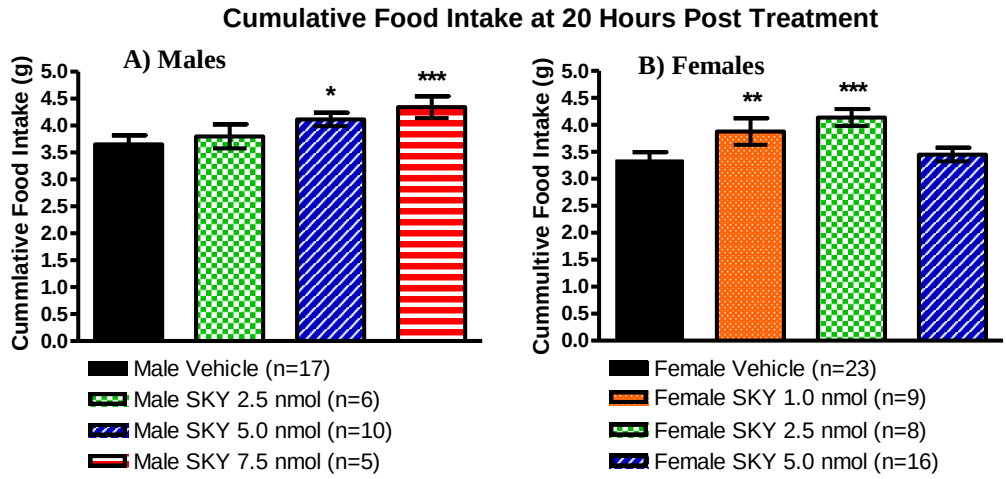


*Fig. 3. Cumulative food intake 20 hours after receiving SKY 2-23-7 in 3 µL vehicle vs 3 µL of vehicle (<1.0% DMSO in water) via ICV administration in both wild type (A) male and (B) female mice. Data shown as mean ± SEM. *p<0.05 **p<0.01 ***p<0.001.*

## Results and Discussion

Both male and female wild type mice underwent surgery to implant a cannula into the lateral ventricle as previously described [9]. After a one week recovery, placement of the cannula was validated via human $PYY_{3-36}$ (Bachem) administration as described by Marsh, et al. [10]. Cannula placement validation was characterized by at least 0.9 g increase in food intake following hPYY treatment versus saline treatment. Mice which passed validation were then administered SKY 2-23-7 after a 1 week washout period. The experiments were performed following a Latin-square paradigm. Vehicle was sterile water with less than 1% DMSO. Mice were housed in TSE PhenoMaster metabolic cage systems (TSE Systems, Berlin Germany). The TSE PhenoMaster system measured the food intake, oxygen uptake, and carbon dioxide production in 15 minute bins following treatment for 96 hours. From the oxygen uptake and carbon dioxide production, the energy expenditure (calories burned) were calculated. Preliminary statistical analyses for compound concentration over time were performed using two-way ANOVA followed by Bonferroni post tests. The analyses showed statistical significant differences for both cumulative food intake and energy expenditure over 24 hours. The data from individual time points were then compared on the bar graphs shown. Individual time point data were analyzed by a one-way ANOVA followed by a Bonferroni post test to compare each treatment group to vehicle treated animals. Statistical significance was considered $p<0.05$.

SKY 2-23-7 displayed a unique pharmacological profile in which it affects male and female mice differently. In male mice, a dose dependent increase in cumulative food intake 20 hours after administration is seen with statistical significance at the 5 and 7.5 nmol doses (Figure 3A). This is consistent with previous reports of mMC4R antagonists increasing food intake [6,9]. In female mice, the 1.0 and 2.5 nmol dose significantly increases food intake (Figure 3B). The same 2.5 nmol dose in males had minimal effect on food intake. Surprisingly, the higher 5 nmol dose in female mice had no effect on food intake which was different from the significant increase in food intake seen in male mice.

Analysis of the energy expenditure in male and female mice also showed sex specific differences. The calories burned in 24 hours was higher in female than in male mice after vehicle administration when normalized for body weight differences ($p=0.001$). When comparing the doses which most significantly affected food intake (7.5 nmol for males; 2.5 nmol for females) opposing effects can be

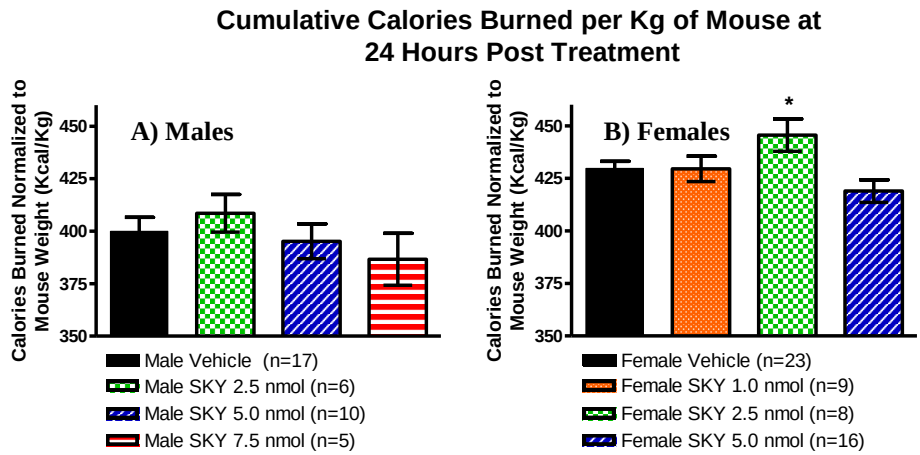


*Fig. 4. Cumulative calories burned normalized to mouse body weight (Kcal/Kg) 24 hours after receiving SKY 2-23-7 in 3µL vehicle vs 3µL of vehicle (<1.0% DMSO in water) via ICV administration in both (A) male and (B) female wild type mice. Data shown as mean ± SEM. *p<0.05.*

seen (Figure 4). The 2.5 nmol dose of SKY2-23-7 in female mice significantly increased the calories burned in 24 hours after ICV administration (Figure 4B). In contrast, the ICV administration of the 7.5 nmol dose of SKY2-23-7 in male mice trended towards decreasing calories burned albeit not significantly (Figure 4A).

These initial findings suggest that ICV administration of SKY 2-23-7 affect male and female mice differently. Due to the observed sex specific responses in food intake and energy expenditure, it can be postulated this compound may have sex specific differences in the side effects of melanocortin ligands related to blood pressure and sexual function. Further probing into a melanocortin sex specific pharmacology could provide a potential avenue to overcome the previously established limitations of melanocortin ligands for their therapeutic use.

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