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Involvement of Both the Forebrain and Hindbrain in the Hypodipsic Effects of Glucagon-like Peptide-1 Receptor Agonists

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Introduction

Glucagon-like peptide-1 (GLP-1) is a peptide produced in the intestines and the brain. It is considered a feeding specific satiety signal, however, evidence shows that it also suppresses water intake independent of its effect on food. There is evidence from our laboratory that both forebrain and hindbrain substrates are involved in the hypodipsic effect of GLP-1 receptor (GLP-1R) agonists. For example, an injection of the GLP-1R agonist, exendin-4, into the fourth ventricle (4V) suppresses overnight intake, indicating that there is hindbrain involvement. Exendin-4 also suppresses intake when injected into the lateral ventricle (LV) of the forebrain, but this requires a lower dose than that needed to affect intake after 4V injection. Thus, there is reason to believe that there is also forebrain involvement. In an initial experiment to determine which specific brain regions are involved, we have generated preliminary evidence that a direct injection of exendin-4 into the nucleus of the solitary tract (NTS) in the hindbrain decreases fluid intake. In addition, differences in the pattern of licking for water was analyzed after NTS injections. Burst analysis is an effective way to examine the root cause of changes in water intake because burst number is affected by variables that change post-ingestive feedback and burst size is affected by the hedonic value of the substance being ingested.

Methods

Subjects. Male, Sprague Dawley rats (325-349 g) were purchased from Harlan Laboratories (Indianapolis, IN). Rats were allowed to habituate to the colony for 1 week before each was implanted with a chronic indwelling cannula aimed at the LV or 4V. After 1 week of recovery, cannula placement was verified. Rats were considered to have proper LV cannula placement if they drank at least 6 ml in response to injection of AngII (10 ng) and rats were considered to have proper 4V cannula placement if their blood glucose doubled after injection of thio-D-glucose (210 µg).

Drug Injections and Intake Measures. Food was removed and rats were administered drugs approximately 30 min prior to lights out. Water intake was measured for the subsequent 24 hr. Licks for water were measured using a contact lickometer.

Data analysis. Statistical testing was performed using Statistica (version 9.0, Statsoft, Tulsa, OK). ANOVA or repeated measures ANOVA was used depending on the experimental design. Significant main or interaction effects ($p<0.05$) were further analyzed using Newman-Keuls post hoc tests. Burst number and average licks per burst were analyzed with a T test for effect of Drug.

Experiment I

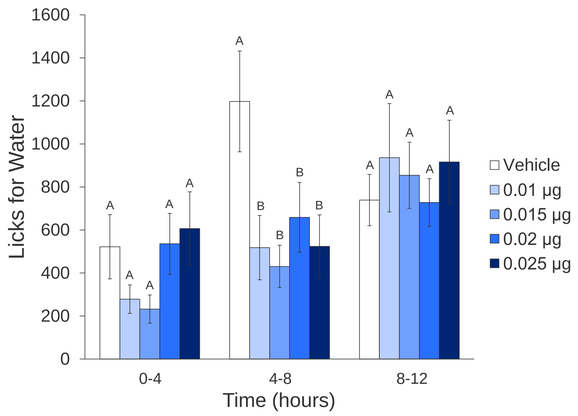

Figure 1. Lateral ventricle dose response of GLP-1R agonist exendin-4 on overnight water intake. Rats injected with any dose of exendin-4 drank significantly less water 4-8 hours after drug injection. Bars with different letters, and within the same bin, are significantly different ($p<0.05$).

Experiment II

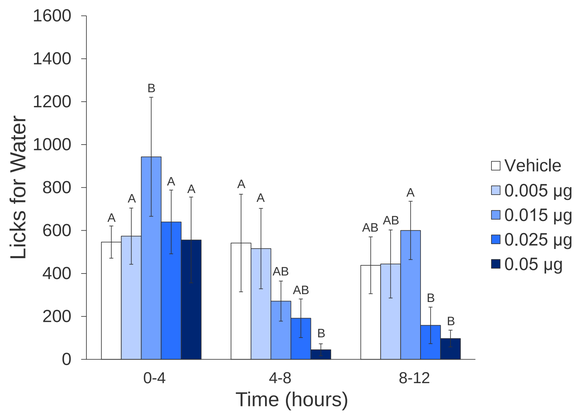

Figure 2. Fourth ventricle dose response of GLP-1R agonist exendin-4 on overnight water intake. Rats injected with the 0.05 µg dose of exendin-4 drank significantly less water 4-8 hours after drug injection. Bars with different letters, and within the same bin, are significantly different ($p<0.05$).

Experiment III

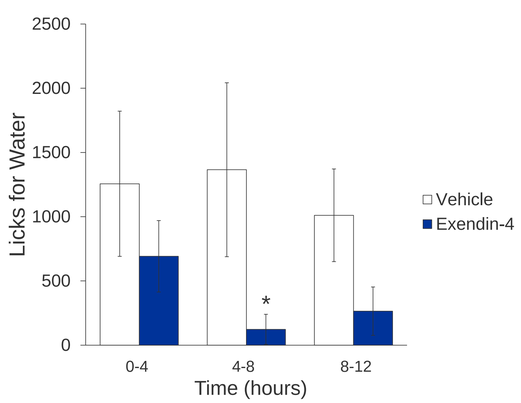

Figure 3. Effect of GLP-1R agonist exendin-4 injection directly into the NTS. Rats injected with a dose of exendin-4 (0.015 µg) that was ineffective after injection into the 4V drank significantly less water 4-8 hours after drug application into the NTS. * indicates statistically different from vehicle within the same time bin.

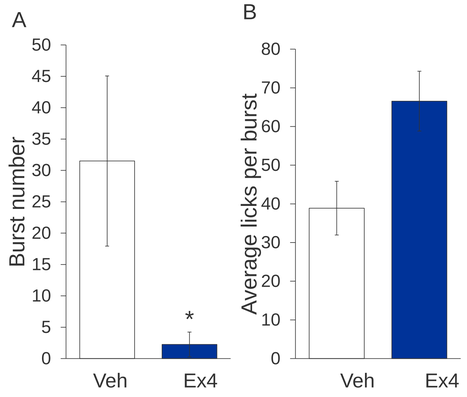

Figure 4. Effect of GLP-1R agonist exendin-4 injection directly into the NTS. Panel A compares the number of bursts between vehicle rats and exendin-4 injected rats, while panel B compares the number likes per burst between the two groups. There were significant group differences ($p<0.05$) in burst number (A), but not in burst size (B).

Summary and Conclusions

- LV injections of GLP-1R agonist exendin-4 suppressed water intake at a very low dose.
- In addition, 4V injections of GLP-1R agonist exendin-4 suppressed water intake, indicating a hindbrain site of action.
- Due to the lower dose necessary to elicit a hypodipsic effect within the LV in comparison to the 4V this suggests an additional forebrain site of action.
- Injections of a subthreshold dose in the ventricle of GLP-1R agonist exendin-4 directly into the NTS suppressed water intake indicating that this region is involved in the hypodipsic effect.
- When lick pattern was analyzed we found that exendin-4 into the NTS decreased burst number, but had no effect on burst size, indicating that the hypodipsic effect was due to changes in post ingestive feedback
- Additional experiments are currently being conducted to determine if a GLP-1R antagonist into either the LV or 4V can block the effects of an agonist.

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