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Research Report

Intra-accumbal administration of shRNAs against CART peptides cause increases in body weight and cocaine-induced locomotor activity in rats

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ABSTRACT

In order to examine the effect of cocaine and amphetamine regulated transcript (CART) peptide depletion in adult rats, CART shRNAs or scrambled control shRNAs were administered bilaterally into the nucleus accumbens (NAc). There was an increase in body weight of the shRNA injected rats compared with the rats injected with the scrambled RNA. This is compatible with the data showing a role for the peptide in body weight and food intake. Also at this time, there was about a two-and-a-half fold increase in cocaine-mediated locomotion in the shRNA injected rats compared to the control rats. This finding is critical support for the hypothesis that endogenous CART peptides in the NAc inhibit the actions of cocaine and other psychostimulants. In immunohistochemical experiments on these same animals, there was a decrease in the staining density of CART peptide in the NAc of the shRNA injected rats. These data show that shRNA can reduce CART peptides in the NAc and that endogenous CART peptides influence body weight and cocaine-induced locomotor activity (LMA).

1. Introduction

Cocaine and amphetamine regulated transcript (CART) peptide neurotransmitters [rlCART 55–102 and 62–102, Rogge et al., 2008] are well known regulators of food intake, body weight, the actions of psychostimulants, other drugs, and other physiologic states (Douglass et al., 1995; Koylu et al., 1998; Kristensen et al., 1998; Lambert et al., 1998; Rogge et al., 2008). The bulk of the evidence for this comes from experiments where exogenous CART peptides are injected into the brains of animals. While the effects of injected, exogenous peptides are clear, the data on the role of endogenous peptides are more limited. Endogenous CART peptides have been examined with injections of anti-CART antibodies, and this has supported a role for CART peptides in feeding (Kristensen et al., 1998; Lambert et al., 1998) and anxiety (Dandekar et al., 2008). CART gene knockout mice have also been prepared (Asnicar et al., 2001; Wierup et al., 2005) and the resulting obesity also supports a role for the peptides in body weight regulation. The discovery of a missense mutation resulting in missorting and improper processing of CART peptide in a family with obesity (del Giudice et al., 2001; Yanik et al., 2006) also supports earlier data linking body weight and CART. However, for drug reward, the knock-out data have been conflicting and have not clearly supported a role for CART peptides in cocaine reward (Couceyro et al., 2005; Moffett et al., 2011; Steiner et al., 2006). In these cases though, the gene knockouts and the genetic mutation are present at all times, and abnormal development

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could play a role in the findings particularly since CART peptides may play a role in development (Brischoux et al., 2001; Brischoux et al., 2002; Dun et al., 2001).

In a larger view of the field, particularly in regard to mechanisms of drug reward, data that are missing include the acute effects of depletion of CART peptides in adult animals. This is important because injected peptides could conceivably act at different brain sites and in a manner that is different from that of endogenous peptides. RNA-interference using small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) have been used to knock down peptide expression without the complications of peptide depletions during development. siRNAs have been used to knockdown CART peptides levels in mice (Jean et al., 2007; Jia et al., 2008), but shRNAs usually provide a longer lasting depletion (Dreyer, 2011). This study was undertaken to provide such data and utilizes short-hairpin RNAs (shRNAs) to deplete the CART peptides after direct injection into the Nucleus Accumbens (NAc).

2. Results

Animals were injected bilaterally with either shRNAs against CART peptides ($n=3$) or the scrambled shRNA (control) ($n=3$) (Fig. 1), into the Nucleus Accumbens (NAc), as described in Section 4. They were returned to the vivarium and their body weights and general conditions were monitored. Because of the extensive, existing data showing that accumbal CART peptide has an effect on body weight (Rogge et al., 2008), this parameter was used as a convenient initial indicator that the shRNA was having an effect. At 8 and 14 weeks there was no significant difference between shRNA and control in percent body weight change from pre-treatment weights, but at 21–23 weeks there was a significant difference between the shRNA and control groups. At 23 weeks, this difference was about 13% (Figs. 2, $^{**}p<0.01$).

Additional experiments were carried out measuring cocaine-induced locomotor activity (LMA) at 8, 14 and 23 weeks (see Section 4). Animals were placed in the activity chambers for 30 min, given a saline injection and monitored for 30 min, and then given a cocaine injection and again monitored for 30 min. The three 30 min periods were referred to as the basal, saline and cocaine periods or components (see Fig. 3). The LMA during these periods was summed and compared (Fig. 4). At 8 and 14 weeks (Fig. 4) there was no difference in cocaine-induced LMA in the shRNA group vs. the scrambled shRNA control group. But at 23 weeks, a time

CART shRNA sequence

1. CCGAGCCCTGGACATCTACTCTGCCGTGG
2. CTCAAGAGTAAACGCATTCCGATCTATGA
3. ATGAGAAGGAGCTGCCAAGGCGGCAACTT
4. ACGCATTCCGATCTATGAGAAGAAGTACG

Scrambled RNA sequence

1. GCACTACCAGAGCTAACTCAGATAGTACT

Fig. 1 – Sequences of shRNAs and Scrambled control RNA.

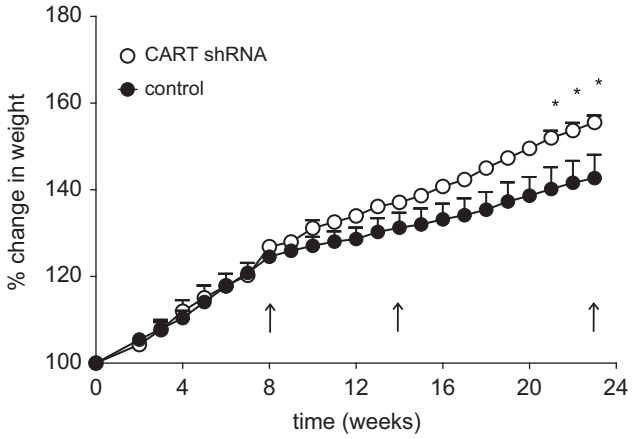

Fig. 2 – Percent change in body weights of rats over time after intra-NAc injections of either CART shRNA or scrambled RNA (control). A two-way ANOVA shows that there is a significant difference between rats injected with CART shRNA ($n=3$) and controls ($n=3$) (F 1, 92=50.09, $^{***}p<0.0001$). The Bonferroni post-tests showed significant differences between treatments at 21–23 weeks ($^{*}p<0.05$). At 23 weeks, rats treated with CART shRNA or control RNA had gained 208±9 g or 170±22 g respectively over their weight at entry into the vivarium. CART shRNA-injected rats show a significant increase in percent weight gain compared to controls. The arrows show the time points when the rats were administered saline and cocaine (i.e. at 8, 14 and 23 weeks).

when the body weights were significantly different between the two groups (Fig. 2), there was also a difference in the cocaine-induced LMA (Figs. 3 and 4, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). Cocaine-induced LMA was increased in the shRNA group compared to the scrambled RNA control group.

The body weight change and cocaine-induced LMA were correlated using all animals for all the experimental periods: 8, 14, and 23 weeks (there are three data pairs for each animal resulting in $n=9$ total, Fig. 3). There was a significant correlation for the animals given the shRNAs, but not for the animals given the scrambled shRNA (controls) (Fig. 5). This is additional evidence that body weight and cocaine-induced LMA are both regulated by CART peptides.

At week 23, after the LMA measurements, the animals were prepared for CART peptide immunocytochemistry (ICC) as described in Section 4. Several regions were examined including the NAc, the dorsal striatum, cerebral cortex and the ventral pallidum (VP). In general, the distribution of CART peptide ICC staining was the same as previously reported in untreated rats (Koylu et al., 1998). There was robust staining in the NAc and VP but little or no staining in the dorsal striatum, and little staining in the cerebral cortex, at the same levels. Non specific staining was assumed to be what was found in the dorsal striatum where there is no CART peptide or mRNA, and this was subtracted from the values from the NAc as described in Section 4. CART peptide staining in the NAc was about 25% lower in the shRNA group (Figs. 6 and 7, $^{*}p<0.05$) compared to the scrambled shRNA control group. CART peptide staining in the VP was not different in the two groups. Thus, the injections of shRNA into the NAc