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Involvement of cocaine- and amphetamine-regulated transcript peptide in the hyperphagic and body weight promoting effects of allopregnanolone in rats

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ABSTRACT

Allopregnanolone (ALLO), a gamma-aminobutyric acid (GABA) type A receptor active neurosteroid, elicits hyperphagic response in rodents. Since GABA-A receptors are present on the peptidergic neurons in the hypothalamus, we were interested in finding out if ALLO and neuropeptide cocaine- and amphetamine-regulated transcript (CART) interact and influence feeding behavior. While subcutaneous ALLO treatment, for a period of 7 days, produced a significant increase in food intake and body weight, pretreatment with subthreshold dose of CART (intracerebroventricular) attenuated both the effects. On the other hand, subcutaneous administration of dehydroepiandrosterone sulfate (DHEAS; GABA-A inhibitor neurosteroid) for a period of 7 days resulted in a significant reduction in food intake and body weight. These effects of DHEAS were potentiated by intracerebroventricular pretreatment with subeffective dose of CART. The brains of ALLO-treated rats were processed for the immunohistochemical analysis of CART immunoreactive elements. ALLO treatment resulted in a significant reduction in CART immunoreactivity in the hypothalamic arcuate, paraventricular and lateral nuclei, and nucleus accumbens shell. The results of the present study suggest that ALLO and CART might interact in the brain, and influence food intake and body weight. However, further investigations are needed to clarify the precise mechanisms by which ALLO modulate feeding behavior.

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1. Introduction

Allopregnanolone (ALLO; 3α-hydroxy-5α-pregnan-20-one) is a potent neuroactive steroid (Robel and Baulieu, 1994, 1995) that exhibits antiepileptic (Lonsdale and Burnham, 2007), hypnotic (Lancel et al., 1997), antidepressant (Khisti et al., 2000) and anxiolytic (Deo et al., 2010) effects. While the gene for ALLO is not expressed in the brain, serum progesterone constitutes the major precursor for the synthesis of ALLO in the several brain regions (Corpechot et al., 1993; Purdy et al., 1991). Peripheral ALLO also contributes in maintaining its levels in the brain (Cheney et al., 1995). Indeed, with a steroidal chemical structure and low molecular weight, ALLO easily penetrates the blood–brain barrier to produce central effects (Lovick, 2008; Wang et al., 2005, 2010). ALLO was detected in the several brain regions including the hypothalamus (Bernardi et al., 1998), and plays an important role in the control of feeding and energy balance. Treatment with ALLO significantly increased the palatable chow consumption not only in food deprived (Reddy and Kulkarni, 1998, 1999), but also in satiated rodents (Chen et al., 1996). ALLO levels were found to be increased in the serum of obese humans (Grosso et al., 2011; Menozzi et al., 2002). In pregnant rats, hyperphagia and weight gain may be attributed to the higher levels of progesterone and ALLO (Douglas, 2011). Olanzapine, an antipsychotic drug with a property to induce hyperphagia and weight gain (Arjona et al., 2004), caused an elevation in ALLO levels in the cerebral cortex of rats (Marx et al., 2000). Gamma-aminobutyric acid (GABA) type A receptors seem to mediate the effect of ALLO on feeding behavior, as ALLO positively modulates GABA-A receptors (Lambert et al., 2003). Indeed, GABA-A receptors are expressed in the hypothalamus (Bäckberg et al., 2004; Blasquez et al., 1994; Fenelon and Herbison, 1995; Moragues et al., 2003), and the antagonist of GABA-A, but not of benzodiazepine, attenuated ALLO-induced palatable chow consumption in fasted animals (Reddy and Kulkarni, 1998, 1999).

Available evidences suggest the colocalization of GABA-A receptors with neuropeptide cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus. While most CART–containing neurons in the hypothalamic arcuate nucleus (ARC) contain proopiomelanocortin (POMC) mRNA (Elias et al., 1998), GABA-A receptor β subunits were noticed on the ARC POMC neurons (Blasquez et al., 1994). Moragues et al. (2003) reported an occasional occurrence of GABA-A receptor ε subunits with the CART neurons of the lateral hypothalamus (LH). Moreover, Bäckberg et al. (2004) demonstrated localization of GABA-A receptors α subunits on CART-containing cell bodies in ventrolateral ARC. These authors further suggested that GABA-A receptors on the ARC neurons may serve as the main target for GABA. A close association between GABA and CART systems is also reported. CART is co-localized with GABA in the ventral tegmental area and nucleus accumbens (Dallvechia-Adams et al., 2002), which is associated with abuse liability of psychostimulant drugs (Fagergren and Hurd, 1999; Roberts, 2005). Moreover, intranuclear injection of CART into the nucleus accumbens shell (AcbSh) attenuated the orexigenic effect of GABA-A receptors agonist, muscimol (Yang et al., 2005). While these evidences raise the possibility that CART may modulate the effects of GABAergic neurosteroids on food intake and body weight, no direct experimental evidences are available.

The present investigation was undertaken to elucidate the role of CART, if any, in the effects of ALLO on feeding behavior. ALLO was injected daily for 7 days, alone and jointly with CART, and food intake and body weight were monitored. Separate groups of rats were subjected to the treatment of dehydroepiandrosterone sulfate (DHEAS), GABA-A inhibitor neurosteroid (Lan and Gee, 1994), alone and concomitantly with CART, to further validate the involvement of GABAergic system in the CART modulated effects of neurosteroids on feeding behavior. Furthermore, a different set of rats was injected daily with ALLO for 7 days, and their brains were subjected to immunohistochemical analysis of CART. The brain regions include ARC, hypothalamic paraventricular nucleus (PVN), periventricular area (PeA), LH and AcbSh. These regions were chosen, since they contain an abundance of CART (Koylu et al., 1997; Upadhya et al., 2012), and play an important role in the regulation of feeding and body weight (Kalra et al., 1999; Konturek et al., 2005; Yang et al., 2005).

2. Results

2.1. *Effect of CART, ALLO or DHEAS treatment on cumulative food intake*

Intracerebroventricular (icv) treatment with CART (1 or 2 μg/rat) resulted in a reduction in food intake below that in the aCSF-treated rats, in a dose dependent manner at 2 h [$F(3, 31)=10.49$, $p<0.0001$], 6 h [$F(3, 31)=26.92$, $p<0.0001$] and 24 h [$F(3, 31)=12.69$, $p<0.0001$] post-injection time-points. As compared to that of control rats, CART at the dose of 1 μg reduced the food intake ($p<0.05$) by 46%, 30% and 22%, while at 2 μg dose, the reduction ($p<0.001$) was 60%, 57% and 45% at 2, 6 and 24 h, respectively. However, at lower dose (0.5 μg/rat, icv), CART did not result in significant anorexic effect ($p>0.05$) (Table 1).

Subcutaneous (sc) administration of ALLO (1 or 2 mg/kg) triggered an increase in food intake, over the vehicle-treated animals, in a dose dependent manner at 2 h [$F(3, 31)=12.67$, $p<0.0001$], 6 h [$F(3, 31)=11.36$, $p<0.0001$] and 24 h [$F(3, 31)=11.60$, $p<0.0001$] post-injection time-points. As compared to that of control rats, ALLO at the dose of 1 mg, increased food intake ($p<0.01$) by 69%, 37% and 26%, while at 2 mg dose, the increment ($p<0.001$) was 96%, 68% and 36% at 2, 6 and 24 h, respectively. However, at lower dose (0.5 mg/kg, sc), ALLO did not produce significant increase in food intake ($p>0.05$) (Table 1).

Contrary to the effects produced by ALLO, DHEAS (4 and 6 mg/kg, sc) produced reduction in food intake below the control values in a dose dependent manner at 2 h [$F(3, 31)=7.8$, $p<0.0001$], 6 h [$F(3, 31)=9.86$, $p<0.0001$] and 24 h [$F(3, 31)=12.69$, $p<0.0001$] post-injection time-points. As compared to that of control rats, DHEAS at the dose of 4 mg decreased food intake ($p<0.05$) by 45%, 38% and 25%, while at 6 mg dose, the reduction ($p<0.001$) was 70%, 55% and 41% at 2, 6 and 24 h, respectively. However, at lower dose (2 mg/kg, sc),