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Lack of effect of ghrelin treatment on melatonin production in rat pineal and Harderian glands

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Abstract

The effects of ghrelin, a peptide hormone secreted from the stomach, on melatonin remain unknown. The aim of the study was to investigate possible ghrelin-melatonin interactions by studying the effect of ghrelin treatment on melatonin production in rat pineal and Harderian glands. Young (9 weeks) and old (20 months) male Wistar rats, maintained under a light:dark cycle regimen of 12:12, were assigned randomly to either a single subcutaneous (s.c.) injection of saline or ghrelin (1 μg/rat or 15 μg/rat) 1 h before sacrifice in the middle of the dark phase, or repeated s.c. saline or ghrelin injections (15 μg/rat), 3, 2 and 1 h before sacrificed in the middle of the dark phase. Neither ghrelin doses (1 μg/rat or 15 μg/rat) nor type of treatment (acute or repeated) influenced melatonin levels or the melatonin synthesizing enzymes *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase activities, either in pineal gland or in Harderian glands. At the concentrations used, ghrelin does not influence melatonin production in rat pineal and Harderian glands, and therefore is not involved in the regulation of melatonin secretion, at least under our experimental conditions.

Keywords: Ghrelin; Melatonin; Pineal gland; Harderian gland; *N*-acetyltransferase; Hydroxyindole-*O*-methyltansferase

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Introduction

Ghrelin is a 28-amino acid peptide hormone secreted from the stomach (Kojima et al., 1999), which acts as a gut-brain peptide with potent stimulatory effects on food intake. Ghrelin has been shown to stimulate growth hormone secretion in rodents (Seoane et al., 2000; Tolle et al., 2001), but also to exert adipogenic and orexigenic effects (Tschöp et al., 2000; Wren et al., 2000; Asakawa et al., 2001). Interestingly, orexin has been shown to influence melatonin synthesis in the rat pineal gland (Mikkelsen et al., 2001), and ghrelin was found to inhibit serotonin (a melatonin precursor) release in the rat hypothalamus (Brunetti et al., 2002). It has been shown in human beings that the β-adrenergic receptors are implicated in the regulation of energy balance (Van Baak, 2001), and it is well established that β-adrenergic receptors play a key role in the regulation of melatonin synthesis. Moreover, evidence reported that exogenous melatonin decreases ghrelin levels in the rat (Mustonen et al., 2001). Together, these observations suggest a relationship between ghrelin and melatonin. Since ghrelin is able to inhibit serotonin, the precursor of melatonin synthesis (Brunetti et al., 2002), we found worth to investigate possible melatonin-ghrelin interactions by studying the effects of ghrelin treatment on melatonin and the associated enzyme activities of *N*-acetyltransferase (NAT) and hydroxyindole-*O*-methyltransferase (HIOMT) in rat pineal glands. The effect of ghrelin on melatonin production in the Harderian glands, orbital structures (Djeridane, 1992, 1994, 1996) considered to be part of the retinal-pineal axis and known to be melatonin producing organs (Djeridane et al., 1998, 1999a,b), has also been examined.

Materials and methods

Animals and experimental design

Male young (5-week-old; 200–300 g body weight) and old (19-month-old; 400–600 g) Wistar rats (JANVIER, Le Genest Saint-Isle, France) on the arrival at the laboratory were used in the experiments. Animals were housed in a chronobiologic animal facility (Enceinte Autonome d'Animalerie, Ref. A 110-SP-6, ESI Flufrance, Arcueil, France) with food and water available ad libitum. The chronobiologic facility was equipped with equispaced, sound-proof, temperature-controlled (21 ± 1.0°C) compartments provided with independent light-dark cycles. The rats were synchronized with a light:dark cycle regimen of 12:12 under a reverse lighting schedule (lights on from 22:00 to 10:00 h) which allowed dark span sampling during the day. The rats were synchronized to this lighting regimen for 4 weeks prior to the experiments.

In a first experiment, young (9 weeks) and old (20 months) rats were assigned randomly (n = 5 animals/group) to a single subcutaneous (s.c.) injection of saline (0.15 M sodium chloride) or ghrelin (Sigma, St. Louis, MO, USA) (1 μg/rat or 15 μg/rat), 1 h before the middle of the dark phase. These doses of ghrelin were chosen since they have already been used in a study dealing with s.c. administration of this drug (Bohan et al., 2003; Enomoto et al., 2003). The rats were sacrificed at 1 h post-injection.

In a second experiment, other sets of young rats (9 weeks) received repeated s.c. injections of saline (n = 5) or ghrelin injections (15 μg/rat) (n = 5), 3, 2 and 1 h before the middle of the dark phase. Rats were sacrificed 1 h after the last ghrelin injection.