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Neuroendocrine regulatory peptide-1 and neuroendocrine regulatory peptide-2 influence differentially feeding and penile erection in male rats: Sites of action in the brain

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ABSTRACT

The effect of NERP-1 and NERP-2, two recently discovered VGF-derived peptides, on feeding and penile erection was studied after injection into the lateral ventricles, the lateral hypothalamus, the arcuate nucleus or the paraventricular nucleus of the hypothalamus. NERP-2 (1–5 nmol), but not NERP-1 (2–4 nmol), increased feeding in a dose-dependent manner when injected into the lateral ventricles or bilaterally into the lateral hypothalamus but not into the arcuate or the paraventricular nucleus. The effect of NERP-2 given into the lateral ventricles was found in the first, but not in the second 60 min after treatment, and was antagonized by SB-408124, an orexin-1 receptor antagonist given into the lateral ventricles or the arcuate nucleus, but not into the paraventricular nucleus. However, SB-408124 was unable to reduce NERP-2-induced feeding when injected bilaterally into the lateral hypothalamus before NERP-2 given also bilaterally into the lateral hypothalamus. In contrast, NERP-1, but not NERP-2, induced penile erection in a dose-dependent manner when injected into the lateral ventricles or the arcuate nucleus, but not into the paraventricular nucleus or the lateral hypothalamus. The pro-erectile effect of NERP-1 was not prevented by the prior injection of $d(CH_2)_5Tyr(Me)^2$-Orn^8-oxytocin or SB-408124 into the lateral ventricles. The present results suggest that while NERP-2 facilitates feeding by acting in the lateral hypothalamus, possibly by increasing orexin activity in the arcuate nucleus, NERP-1 facilitates penile erection by acting in the arcuate nucleus with a mechanism not related to orexin or oxytocin.

1. Introduction

Neuroendocrine regulatory peptide-1 and neuroendocrine regulatory peptide-2 (NERP-1 and NERP-2) are two novel biologically active peptides recently identified in the middle portion of the 617 amino acid residue VGF protein in the human medullary thyroid carcinoma TT cells [1] and that have been added to the list of biologically active peptides derived by the proteolytic processing of VGF by endopeptidases acting at basic amino acid residue pairs (see [2–4] and references therein). In the rat NERP-1 and NERP-2 correspond to the 25 amino acid residue fragment $VGF_{285\text{-}309}$ and the 38 amino acid residue fragment $VGF_{313\text{-}350}$, respectively [1]. Both peptides are C-terminal amidated, and C-terminal amidation is essential for eliciting their ability to suppress vasopressin release induced by the intracerebroventricular (I.C.V.) injection of hypertonic saline or angiotensin II [1,5]. These peptides are abundant in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON), where they are frequently colocalized with vasopressin and, although less frequently, with oxytocin [1,5]. In addition to the PVN and SON, NERPs are also present in other brain areas such as the arcuate nucleus (ARC), the lateral hypothalamus (LH), the ventral tuberomammillary nucleus, the supraoptic retrochiasmatic nucleus, the cerebral cortex, the pituitary and thyroid glands, and in the periphery at the level of the gastrointestinal tract [6–8]. In particular, a decrease of the concentration of NERPs was found in the cortex of sample tissues from patients affected by Parkinson's disease and Alzheimer's disease, suggesting that a loss of NERPs may lead to these neurodegenerative diseases [6]. Recent experiments have shown that NERP-2, but not NERP-1, can be considered a novel member of the subset of feeding-regulatory peptides functioning in the hypothalamus [9]. Accordingly, the I.C.V. administration of NERP-2, but not of NERP-1, at doses that stimulate feeding behavior, induced cFOS protein, a marker in LH orexin-immunoreactive neurons, and feeding itself increased NERP-2 immunorectivity in LH orexin neurons. This suggests that NERP-2 but not NERP-1 may activate orexinergic neurons to release orexin in brain areas important for the induction of feeding behavior, most probably through the activation of the potent feeding-inducing peptide neuropeptide Y. Accordingly, (1) NERP-2 did not increase food intake in orexin-deficient mice [9] and (2) NERP-2-induced feeding was abolished by the

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I.C.V. administration of anti-orexin immunoglobulins (IgG) or of a selective orexin-1 receptor antagonist, as well by the I.C.V. administration of selective antagonists of neuropeptide Y receptors of the type Y1 and Y5 [9]. We have recently found that a few VGF C-terminal peptide fragments induce penile erection when administered into the PVN of male rats, apparently by increasing the activity of oxytocinergic parvocellular neurons projecting to extra-hypothalamic brain areas and the spinal cord [10,11]. Interestingly, NERPs are found colocalized with oxytocin not only in magnocellular but also parvocellular oxytocinergic neurons in the PVN [1]. In order to provide further evidence for a role of NERPs in the control of feeding and of VGF-derived peptides in the control of penile erection, the effect of the injection of NERPs into several hypothalamic nuclei of male rats on feeding and on penile erection was studied. The possible interaction of NERPs with other neuropetides involved in these behaviors was also studied. For comparison, the effect of ghrelin, a potent inducer of feeding (see [12]), on this response is also reported.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) (Charles River, Como, Italy) were used in all the experiments. The animals were caged in groups of four to six at 24 °C, humidity 60%, lights on from 07:00 to 19:00 h with water and standard laboratory food ad libitum. The experiments were performed between 09:00 and 13:00 h. All experiments were carried out in accordance with the guidelines of the European Communities Directive of 24 November 1986 (86/609/EEC) and the Italian Legislation (D.P.R. 116/92).

2.2. Drugs and peptides

Rat NERP-1 ($VGF_{285-309}$) (H-Leu-Glu-Gly-Ser-Phe-Leu-Gly-Gly-Ser-Glu-Ala-Gly-Glu-Arg-Leu-Leu-Gln-Gln-Gly-Leu-Ala-Gln-Val-Glu-Ala-NH2 trifluoroacetate salt), rat NERP-2 ($VGF_{313-350}$) (Pyr-Ala-Glu-Ala-Thr-Arg-Gln-Ala-Ala-Ala-Gln-Glu-Glu-Arg-Leu-Ala-Asp-Leu-Ala-Ser-Asp-Leu-Leu-Leu-Gln-Tyr-Leu-Leu-Gln-Gly-Gly-Ala-Arg-Gln-Arg-Asp-Leu-Gly-NH2 trifluoroacetate salt), mouse/rat ghrelin [H-Gly-Ser-Ser(octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Lys-Ala-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-OH trifluoroacetate salt] and $d(CH_2)_5Tyr(Me)^2$-Orn^8-oxytocin were purchased from Bachem AG (Bubendorf, CH); SB-408124 (*N*-(6,8-difluoro-2-methyl-4-quinolinyl)-*N*′-[4-(dimethylamino)phenyl]urea) from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were from available commercial sources.

2.3. Microinjections into the lateral ventricles (I.C.V.), into the paraventricular nucleus (PVN), arcuate nucleus (ARC) and lateral hypothalamus (LH)

Microinjections were performed through stainless steel guide cannulas (22 gauge) stereotaxically implanted (Stoelting Co., Wood Dale, IL, USA) in the skull of male rats under chloral hydrate anaesthesia (400 mg/kg i.p.), aimed unilaterally at the lateral ventricle (coordinates: 1.0 mm posterior to bregma; 1.5 mm lateral to midline; 2.0 mm ventral to the skull surface), or at the PVN (coordinates: 1.8 mm posterior to bregma; 0.4 mm lateral to midline; 2.0 mm ventral to the skull surface), or at the ARC (coordinates: 2.8 mm posterior to bregma; 0.3 mm lateral to midline; 2.0 mm ventral to the skull surface), or bilaterally at the LH (coordinates: 2.8 mm posterior to bregma; 1.5 mm lateral to midline; 2.0 mm ventral to the skull surface) [13]. For microinjections I.C.V. and into the ARC or I.C.V. and into the PVN of the same rat, a chronic guide cannula was implanted as described above at the following coordinates, 0.2 mm anterior to bregma, 0.4 mm lateral to midline, 2.0 mm ventral to the dura, according to [14]. This allowed microinjections I.C.V. and into the ARC or I.C.V. and into the PVN of the same rat, respectively, simply by lowering the same microinjection cannula to the required depth (see Results, Section 3.3). Animals were given 7 days to recover from surgery for food intake experiments and 3 days for penile erection studies, respectively. For food intake studies, only those animals that had not showed progressive body weight loss after surgery were used; each rat was used only once and only in one experiment. When microinjections were performed, the microinjection cannula that extended 1.5 mm below the bottom tip of the guide cannula for I.C.V. injections (1.0 when coordinates from [14] were used), 6.0 mm for PVN injections (5.5 when coordinates from [14] were used), 8.0 mm for ARC injections (7.5 when coordinates from [16] were used), and 6.5 mm for LH injections was connected by polyethylene tubing to a 10 μl Hamilton syringe driven by a Stoelting microinfusion pump. NERP-1, NERP-2 or ghrelin dissolved in saline or saline alone (controls) was injected I.C.V. in a volume of 10 μl, unilaterally into the PVN or the ARC in a volume of 0.3 μl or bilaterally into the LH in a volume of 0.5 μl per side. When SB-408124 was used, the compound was dissolved in DMSO and diluted with distilled water and administered to rats as described above 30 min before NERP-1 or NERP-2. Control rats were treated with the same volume of vehicle 30 min before NERP-1 or NERP-2. When $d(CH_2)_5Tyr(Me)^2$-Orn^8-oxytocin was used, the peptide was dissolved in saline and administered to rats 15 min before NERP-1 or NERP-2. Control rats were treated with the appropriate volume of saline 15 min before NERP-1 or NERP-2. All injections were performed in a period of 2 min. After each injection, the microinjection cannula was left into the injection site for additional 30 s to allow an optimal spreading of the injected compound.

2.4. Behavioral studies

For feeding experiments, rats were placed individually into Plexiglas cages (30×30×30 cm). After a 30-min habituation period, NERP-1, NERP-2, ghrelin or saline (controls) was injected into the lateral ventricles or into the hypothalamic nuclei (PVN, ARC, LH) of rats implanted with chronic guide cannulas as described above. When SB-408124 was used, the compound or its vehicle (10% DMSO in distilled water) was given to rats 30 min before NERP-2 or saline injected into the same hypothalamic nuclei. Controls rats were treated with the appropriate volume of vehicle 30 min before saline injection into the same brain sites. Immediately after the injection of NERP-1, NERP-2 or ghrelin, a small cup with pre-weighted 10 g of standard laboratory food pellets was introduced into the cage and rats allowed to feed. Feeding was monitored for 60 or 120 min, depending on the experiment and at the end of this period the residual food was carefully collected and weighed to the nearest 0.01 g as already described [15,16].

For penile erection experiments, NERP-1 and NERP-2 were injected I.C.V. or into the hypothalamic nuclei (PVN, ARC, LH) of rats implanted with chronic guide cannulas as described above. When SB-408124 was used, the compound dissolved in 10% DMSO in distilled water was given I.C.V. to rats 30 min before NERP-1 or saline. When $d(CH_2)_5$-$Tyr(Me)^2Orn^8$-oxytocin was used, the compound was given I.C.V. to rats 15 min before NERP-1 or saline. Control rats were treated with the appropriate volume of vehicles. After treatments, rats were observed for 60 min in order to count penile erection episodes. Penile erections were scored when the penis emerged from the penile sheath, which was usually accompanied by penile grooming and hip flexions as already described [15].

2.5. Histology

In order to verify the correct injection into the lateral ventricles, at the end of the experiments rats were injected with 10 μl of the vital colorant methylene blue, then killed and visually inspected. Only those animals found to have the colorant distributed correctly in the cerebral ventricles were considered for the statistical evaluation of the results. When microinjections were performed into the PVN,