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Activation of Hypocretin-1/Orexin-A Neurons Projecting to the Bed Nucleus of the Stria Terminalis and Paraventricular Nucleus Is Critical for Reinstatement of Alcohol Seeking by Neuropeptide S

Massimo Ubaldi, Antonio Giordano, Ilenia Severi, Hongwu Li, Marsida Kallupi, Giordano de Guglielmo, Barbara Ruggeri, Serena Stopponi, Roberto Ciccocioppo, and Nazzareno Cannella

ABSTRACT

BACKGROUND: Environmental conditioning is a major trigger for relapse in abstinent addicts. We showed that activation of the neuropeptide S (NPS) system exacerbates reinstatement vulnerability to cocaine and alcohol via stimulation of the hypocretin-1/orexin-A (Hcrt-1/Ox-A) system.

METHODS: Combining pharmacologic manipulations with immunohistochemistry techniques, we sought to determine how NPS and Hcrt-1/Ox-A systems interact to modulate reinstatement of alcohol seeking in rats.

RESULTS: Intrahypothalamic injection of NPS facilitated discriminative cue-induced reinstatement of alcohol seeking. This effect was blocked by the selective Hcrt-1/Ox-A antagonist SB334867 microinjected into the hypothalamic paraventricular nucleus (PVN) or into the bed nucleus of the stria terminalis (BNST) but not into the ventral tegmental area or the locus coeruleus. Combining double labeling and confocal microscopy analyses, we found that NPS-containing axons are in close apposition to hypothalamic Hcrt-1/Ox-A positive neurons, a significant proportion of which express NPS receptors, suggesting a direct interaction between the two systems. Retrograde tracing experiments showed that intra-PVN or intra-BNST red fluorobead unilateral injection labeled bilaterally Hcrt-1/Ox-A somata, suggesting that NPS could recruit two distinct neuronal pathways. Confirming this assumption, intra-BNST or PVN Hcrt-1/Ox-A injection enhanced alcohol seeking similarly to hypothalamic NPS injection but to a lesser degree.

CONCLUSIONS: Results suggest that the Hcrt-1/Ox-A neurocircuitry mediating the facilitation of cue-induced reinstatement by NPS involves structures critically involved in stress regulation such as the PVN and the BNST. These findings open to the tempting hypothesis of a role of the NPS system in modulating the interactions between stress and environmental conditioning factors in drug relapse.

Keywords: Addiction, Cue, Hypocretin, Neuropeptide S, Orexin, Relapse, Stress

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The occurrence of stressful life events concurrently with the exposure to environmental stimuli predictive of drug availability is critical to increased relapse risk in abstinent addicts (1–3). Development of efficacious anti-relapse treatments requires a deep understanding of the interaction between stress and environmental conditioning factors in triggering relapse.

Over the recent years, it has been demonstrated that the hypothalamic hypocretin-1/orexin-A (Hcrt-1/Ox-A) system may play a unique role in integrating responses triggered by stress and conditioning factors. For instance, it has been shown that activation of this peptidergic transmitter system mediates arousal and goal-oriented behavior (4–6). Hcrt-1/Ox-A mediates reinstatement of morphine place preference (7) and cocaine and alcohol seeking induced by re-exposure to contextual and contingent cues (8–11). Additionally, a role for Hcrt-1/Ox-A in stress-induced relapse has also been proposed, as the selective orexin/hypocretin receptor type 1 (OX1) antagonist SB334867 blocked stress-induced reinstatement of cocaine and morphine seeking (12,13).

In previous studies, we demonstrated that the pro-reinstatement activity of Hcrt-1/Ox-A can be triggered by upstream activation of the neuropeptide S (NPS) system (14–16). NPS is a neurotransmitter produced in the pericoerulear zone of the brainstem and is released in the lateral hypothalamus (LH) where it activates its cognate $G_{q/s}$ protein-coupled receptor named neuropeptide S receptor (NPSR) (17,18). When administered into the LH, NPS enhances reinstatement of alcohol and cocaine seeking triggered by environmental conditioning factors. This effect is blocked by peripheral administration of the OX1 antagonist SB334867, which

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suggests an important interaction between these two peptidergic systems (15,16,19). In addition, NPS facilitates cocaine reinstatement with mechanisms mimicking, at least in part, the effect of stress and that may involve the corticotropin releasing factor and the Hcrt-1/Ox-A systems as well (20–22). These evidences suggest a possible scenario where NPS acting as an upstream modulator of the Hcrt-1/Ox-A neurons may regulate the concerted activation of specific Hcrt-1/Ox-A neurocircuitries that are then responsible for relapse to drug seeking. Here, combining brain-specific microinjection of NPS and SB334867 with morphologic analysis using double labeling confocal microscopy and retrograde tracing techniques, we identified the Hcrt-1/Ox-A neurocircuitries that, when activated by NPS, facilitate discriminative cue-induced reinstatement of alcohol seeking in rats trained to discriminate between alcohol and water paired environments. Our main finding is that NPS facilitates reinstatement of seeking behavior through specific activation of Hcrt-1/Ox-A neurons projecting to the paraventricular nucleus (PVN) and the bed nucleus of the stria terminalis (BNST), two areas classically linked with stress regulation. This may represent an important neuroanatomical substrate for the integration of conditioning and stress-related responses in drug relapse.

METHODS AND MATERIALS

Detailed methods are given in Supplement 1.

Animals

Male Wistar rats were used. Procedures adhered to the European Community Council Directive and the National Institutes of Health Guidelines for Care and Use of Laboratory Animals.

Intracranial Surgery

Guide cannulae for drug injections were stereotaxically aimed at the following regions: LH [we previously demonstrated that NPS stimulates Hcrt-1/Ox-A neurons when microinjected into the perifornical and lateral hypothalamic regions of the rat tuberal hypothalamus (15,19); therefore, in the present work, we used stereotaxic coordinates, allowing us to diffuse NPS into both areas], locus coeruleus (LC), ventral tegmental area (VTA), PVN, and BNST. Cannulae were always implanted bilaterally, except for the injection of the retrotracer in experiment 5 where they were implanted unilaterally in the PVN and BNST.

Drug Injection and Histological Analysis

The following compounds were used for pharmacologic experiments: the selective nonpeptide OX1 antagonist SB334867, NPS, and Hcrt-1/Ox-A.

Operant Training and Alcohol Self-Administration

Rats were trained to self-administer 10% alcohol (vol/vol) using a saccharine fading procedure adapted from Weiss *et al.* (23) in 30-minute daily sessions on a fixed-ratio 1 schedule of reinforcement.

Discriminative Cue-Induced Reinstatement of Alcohol-Seeking Behavior

Experiments consisted of three phases: discrimination, extinction, and reinstatement.

Discrimination. Rats were trained to discriminate between 10% alcohol and water. Discriminative and discrete stimuli predictive of alcohol (S^+) versus water availability (S^-) were presented during the alcohol and water self-administration sessions, respectively (10 each).

Extinction Phase. After the last conditioning day, rats were subjected to 30-minute extinction sessions for a minimum of 10 consecutive days and until they produced less than 12 responses on the active lever for at least 2 consecutive days.

Reinstatement Test. Reinstatement tests began the day after the last extinction session. Test sessions were identical to the discrimination phase, except that alcohol and water were not made available. The effect of NPS and/or SB334867 and Hcrt-1/Ox-A on discriminative cue-induced reinstatement of alcohol seeking was evaluated in a Latin square within-subject counterbalanced design.

Experiment 1: Effect of Intra-VTA SB334867 Administration on NPS-Induced Enhancement of Discriminative Cue-Induced Reinstatement of Alcohol Seeking

Rats (n = 10) received 5 μg of SB334867 or an equivalent volume of its vehicle into each VTA. Five minutes later, animals received .25 nmol of NPS or vehicle into each LH. Immediately after injections, rats were placed in the operant cages and tested for discriminative cue-induced reinstatement of alcohol seeking.

Experiment 2: Effect of Intra-LC SB334867 Administration on NPS-Induced Enhancement of Discriminative Cue-Induced Reinstatement of Alcohol Seeking

Rats (n = 10) were treated as in experiment 1, except that SB334867 (or its vehicle) was injected bilaterally into the LC.

Experiment 3: Effect of Intra-PVN SB334867 Administration on NPS-Induced Enhancement of Discriminative Cue-Induced Reinstatement of Alcohol Seeking

Rats (n = 10) were treated as in experiment 1, except that SB334867 (or its vehicle) was injected bilaterally into the PVN.

Experiment 4: Effect of Intra-BNST SB334867 Administration on NPS-Induced Enhancement of Discriminative Cue-Induced Reinstatement of Alcohol Seeking

Rats (n = 10) were treated as in experiment 1, except that SB334867 (or its vehicle) was injected bilaterally into the BNST.