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Brief Communication

Intra-ventrolateral preoptic nucleus injection of γ-aminobutyric acid induces sedation in rats

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Abstract: The ventrolateral preoptic nucleus (VLPO) plays a critical role in regulating and maintaining sleep-awake cycle. It receives both excitatory and inhibitory inputs and regulates the activity of tuberomamillary nucleus and other monoaminergic nuclei, which in turn determines the alternation between wakefulness and non-rapid eye movement sleep. Although a previous study has shown that systematic administration of GABAergic anesthetic agents activated VLPO neurons, which is believed to be responsible for the sedative effects of these agents, it is unknown whether a direct administration of γ-Aminobutyric acid (GABA) into the VLPO can induce sedation. Here we report that rats that received intra-VLPO infusion of GABA demonstrated sustained reduction in locomotion, most significantly during the 10-40th min period after infusion. Conversely, rats that received intra-VLPO infusion of noradrenaline demonstrated a sustained increase in locomotion from 20th min after infusion. By contrast, no appreciable change was observed in rats that received intra-VLPO infusion of glycine. This result demonstrates that exogenous GABA may activate sleep-active neurons in the VLPO and promote sedation.

Keywords: Ventrolateral preoptic nucleus, GABA, sedation

Introduction

Sleep mechanism has been intensely studied over the past decades and several neuronal pathways have been proposed. Among the discreet regions of the brain involved in the pathways, the ventrolateral preoptic nucleus (VLPO) is found to play a critical role in regulating and maintaining the sleep-awake cycle [1]. The VLPO is considered a part of the basal forebrain and is composed of neuronal cells located in the anterior hypothalamus. During non-rapid eye movement sleep (NREM) the VLPO neurons are active and release the inhibitory neurotransmitters GABA and galanin, which inhibit the monoaminergic cell groups in the locus coeruleus, the raphe nucleus, and the tuberomammillary nucleus [1, 2] that are involved in wakefulness. γ-Aminobutyric Acid (GABA)-containing neurons in the VLPO are also under tonic inhibition from the arousal systems. Reciprocally, transmitters used by these systems, including acetylcholine (ACh), serotonin and noradrenaline (NA), exert an inhibitory action on the VLPO neurons [2-4]. Therefore, multiple neurotransmitters have been revealed to mediate the excitatory and inhibitory inputs to the VLPO nucleus. Although GABA is the most abundant inhibitory neurotransmitter in the brain, the connection between the $GABA_A$ receptors in the VLPO and the sedative response to GABAergic agents is still obscure.

A previous study demonstrated that VLPO neurons also mediate the sedative effects of GABAergic anesthetic agents; systemic administration of GABAergic agents increased the activity of VLPO neurons and induced sedation in rats [5]. In this study, we examined the effect of direct administration of GABA into the VLPO on locomotion of rats, an indication of sedation.

Materials and methods

Animals

Male Sprague-Dawley rats (n = 19) weighing 180-200g were obtained from Taconic Farms (Germantown, N.Y., USA). Rats were individually housed in ventilated cages with controlled hu-

midity and temperature (22°C), a 12:12-h light/dark cycle (on 07:00, off 19:00), and free access to food and water. The experimental procedures were performed during the light cycle. All experiments were performed in accordance with the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey, New Jersey.

Stereotaxic surgery

To test the effect of intra-VLPO administration of GABA and glycine, 10 animals were used in this experiment. To validate our system, another 9 rats were used to test the effects of noradrenaline on locomotor activity. Rats were anesthetized intraperitoneally with ketamine/xylazine (80 mg/10 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf, USA). An incision (8-10 mm) was made in the skin over the skull, and the wound margin was infiltrated with lidocaine (1%). Each animal was implanted with one bilateral guide cannulae (23 gauge; Plastics One), aimed at the VLPO. The stereotaxic coordinates relative to bregma and skull surface (Paxinos and Watson, 2007) were as follows: 0.12 mm posterior to bregma, 0.9 mm mediolateral, 8.8 mm ventral to the skull surface. Cannulae assemblies were secured in place with dental cement and two screws. A stainless steel dummy DBL (28-gauge; Plastics One) was put in place at the time of surgery and removed at the time of testing. Following surgery, rats were housed in individual rectangular plastic cages, located side by side in order to prevent the influence of chronic stress on performance due to isolation, with free access to food and water for at least 7 days.

Seven days after surgery, animals were taken from the colony, brought to the experimental room, and handled for 5 min per day until the experimental day. During this phase, animals became accustomed to the experimenter, the experimental room, and to the manipulation procedure with a total of four to five sessions to decrease the novelty activating effects of the manipulations.

Drugs infusion and behavioral procedures

Noradrenaline bitartrate was obtained from Sicor Pharmaceuticals (Irvine, CA). Glycine and γ-Aminobutyric Acid were purchased from Sigma Chemical (St. Louis, MO). On the test day, rats were again taken from the colony room and brought to the experimental room 1 hour prior to the start of the session. Following this habituation period, obstructers were gently removed and 28-gauge internal cannulae (Plastics One, Roanoke, VA, USA) were inserted bilaterally to the VLPO. Rats were placed in the observation chamber (16in x 16in x 16in, Coulbourn Instrument, Whitehall, PA). Rats were acclimated to the activity monitors for 15 min prior to drug injections to minimize effects of novelty. Saline vehicle, GABA (1 or 10 mM), glcyine (10 mM) and noradrenaline (3 mM) in a total volume of 0.2 μl was infused over 1 min into the VLPO of rats via internal cannulae connected to a Hamilton 1.0 μl syringe driven by a syringe pump (Harvard Instruments, Holliston, Massachusetts, USA). The order of treatments was partially counterbalanced such that vehicle was given first, followed by injections of drug, with doses administered in random order.

Locomotion activities were automatically recorded using TruScan 2.0 software (Coulbourn Instruments, Whitehall, PA) at 5 min intervals for 60 min (including the first 15 min prior to drug injections). Total movements during the first 5 minutes were recorded respectively as the baseline. Locomotion data in each rat were normalized to the baseline in order to compare results among different treatment groups.

Histological verification of cannulae placements

At the end of the experiments, rats were overdosed with ketamine/xyzaline and transcardially perfused with cold-buffered saline followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4); brains were removed, postfixed (2 hours, at 4°C) in the same fixative solution and cryoprotected (overnight at 4°C, 20% sucrose in 0.1 M phosphate buffer, pH 7.4). Serial 30-μm coronal sections across the cannulation site were prepared using a freezing microtome (Microm HM550, Walldorf, German). Data were utilized only when the correct placement of the cannulae had been microscopically confirmed on cresyl violet-stained brain sections.

Data analysis

The total movements across the 60-min sessions was analyzed using two-way analysis of

variance with the repeated measure (RM ANOVA) with time as the within-subject factor and treatment as the between factor. *Post hoc* comparisons were conducted by Tukey's test. It was determined that $p < 0.05$ was statistically significant.

Results

We first tested the effects of GABA injection into the VLPO. Two animals in this experiment showed cannulae tip placements outside the VLPO. These animals were excluded from the statistical analysis. The cannulae tip placements for the rats included in this experiment are depicted in **Figure 1**. As depicted in **Figure 2**, intra-VLPO infusion of GABA (10 mM) induced sustained reduction in movement. Two-way ANOVA revealed a significant main effect of treatment [$F_{1,143} = 18.46$, $p < 0.001$] and a main effect of time [$F_{11,143} = 27.79$, $p < 0.001$] with significant main effect of GABA treatment × time interaction [$F_{11,143} = 3.18$, $p < 0.001$]. *Post hoc* comparisons revealed most significant difference was observed during the 25-55th min period (*vs*. saline vehicle, all $p < 0.05$).

We next tested the effects of glycine, another major inhibitory neurotransmitter in the central nervous system. In contrast to GABA, rats treated with 10 mM glycine demonstrated no observable changes in movement compared to saline group (**Figure 3**). Two-way ANOVA showed a significant decrease in the total movements of 5-min intervals over time [$F_{11, 143} = 9.65$, $p = 0.94$], but no significant main effect of treatment [$F_{1, 143} = 0.0052$, $p > 0.05$] or treatment × time interaction [$F_{11, 143} = 0.70$, $p = 0.73$].

Finally, to validate our system, we measured the effects of noradrenaline, an important transmitter of wakefulness [6]. One animal in this experiment showed cannulae placement outside the VLPO. This animal was excluded from the statistical analysis. In agreement with previous reports, rats treated with 3 mM noradrenaline demonstrated sustained movement activity. Two-way ANOVA revealed significant main effects of treatment [$F_{1,132} = 12.79$, $p = 0.004$] and time [$F_{11,132} = 11.47$, $p < 0.001$] with significant main effect of noradrenaline treatment × time interaction [$F_{11,132} = 7.18$, $p < 0.001$]. *Post hoc* comparisons revealed significant difference was observed from the 35th min and sustained to 60th min (*vs*. saline vehicle, all $p < 0.05$, **Figure 4**).

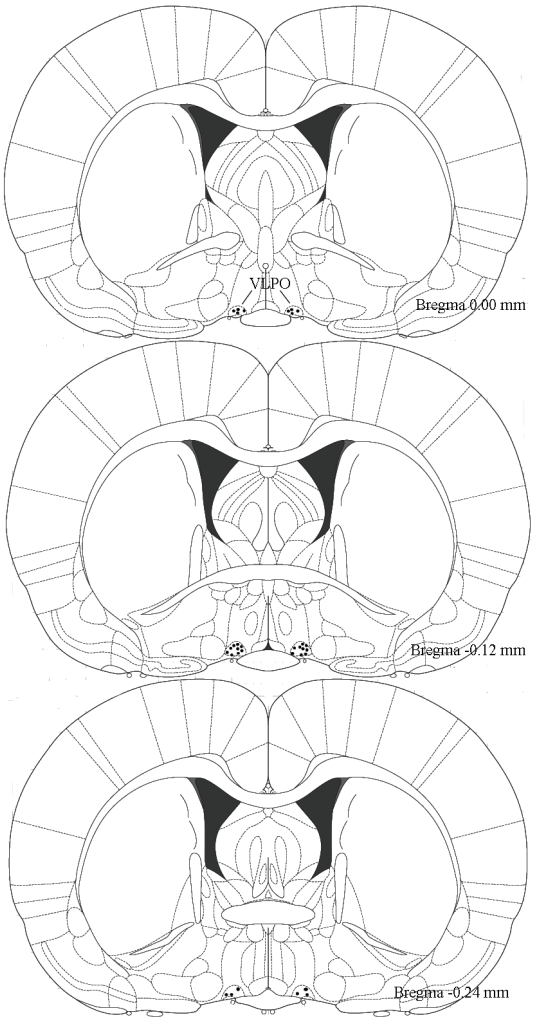

Figure 1. Illustration of injection cannula tips (black dots) within the VLPO from the brains of rats used for the behavior experiments summarized in figures 2, 3 and 4. The schematic brain sections are from the atlas of Paxinos and Watson (adapted from Paxinos and Watson, 2007). The numbers indicate mm from bregma.

Discussion

Here, we show that direct injection of GABA into the VLPO reduced rat locomotion over time, which suggests that the exogenous GABA may directly activate sleep-active neurons in the VLPO and promote sedation.

Evidences from several labs including our own have revealed two major types of neurons in the VLPO: the majority (69%) are inhibited by noradrenaline (NA-inhibited neurons) and the

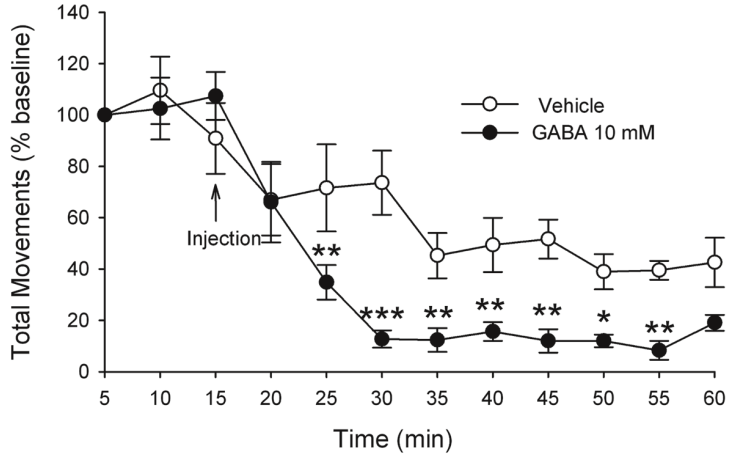

Figure 2. Intra-VLPO infusion of 10 mM GABA induces sustained reduction in total movements in rats. Total movement counts obtained during the first 5 minutes were recorded as the baseline. Locomotor activity counts obtained of 5-min interval in each rat were normalized to the baseline. Data are illustrated as mean ± S.E.M. Asterisks indicate significant differences between groups as revealed by *post hoc* Tukey comparisons following two-way RM ANOVA. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$; GABA compared to saline vehicle (n = 8 per group).

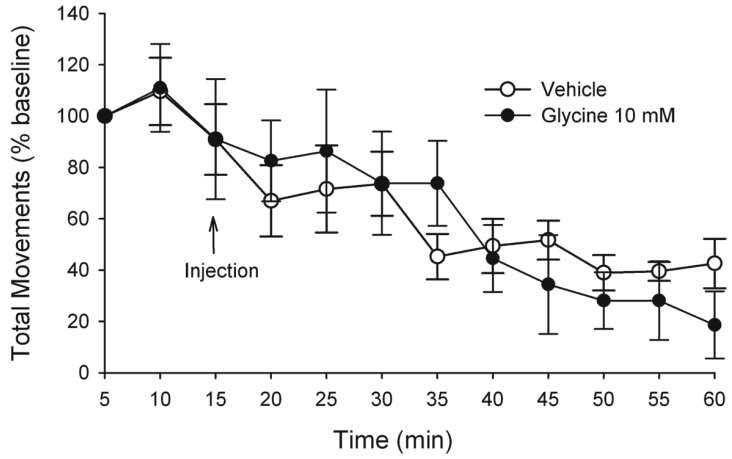

Figure 3. Intra-VLPO infusion of 10 mM glycine does not affect locomotor activity in rats. Total movement counts obtained during the first 5 minutes were recorded as the baseline. Locomotor activity counts obtained of 5-min interval in each rat were normalized to the baseline. Data are illustrated as mean ± S.E.M., n = 8 per group.

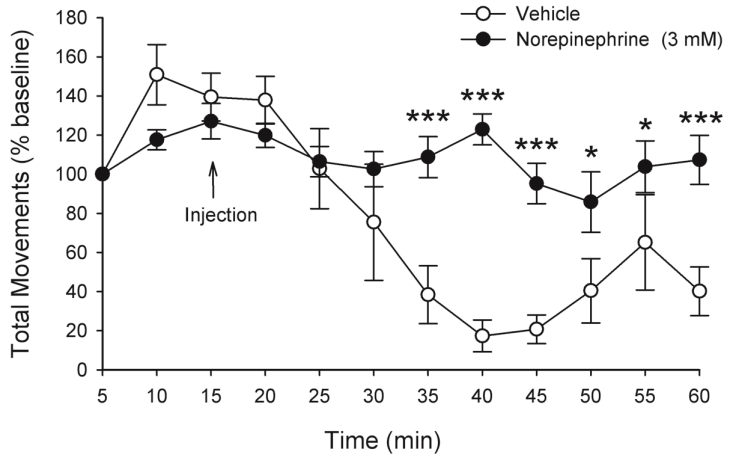

Figure 4. Intra-VLPO infusion of noradrenaline induces sustained locomotor activity in rats. Total movement counts obtained during the first 5 minutes were recorded as the baseline. Locomotor activity counts obtained of 5-min interval in each rat were normalized to the baseline. Data are illustrated as mean ± S.E.M. Asterisks indicate significant differences between groups as revealed by *post hoc* Tukey comparisons following two-way RM ANOVA. ***$p < 0.001$, *$p < 0.05$; noradrenaline compared to saline vehicle (n = 8 per group).

minority are excited by NA (NA-excited neurons) [4, 7]. The NA-inhibited neurons containing GABA are sleep-promoting neurons and project to the histamine-releasing neurons in the tuberomammillary nucleus[4, 8-10]. When excited, these neurons release GABA into the tuberomammillary nucleus, thus inhibiting the activity of this arousal-producing nucleus and

reducing the release of histamine and elicit the onset of sleep. In harmony with this idea was the current finding that intra-VLPO infusion of noradrenaline successfully reproduced sustained wakefulness in the rats, which validated our system. This result is consistent with that noradrenaline inhibited VLPO neurons both in the in vivo and in vitro settings [4, 6, 7, 11].

The major finding of the current study is that intra-VLPO infusion of GABA, but not glycine significantly reduced locomotion over time in rats. This result suggests that exogenous GABAergic agents may excite sleep-active neurons in the VLPO and promote sedation. This is in general agreement with a previous report that systematic administration of GABAergic agents activated VLPO neurons [5]. The mechanism under this observation is supported by the result of an *in vitro* study from our lab which showed that GABAergic agents such as propofol and muscimol led to increased glutamate release in the VLPO neuron [12]. Because chloride channel and $GABA_A$ receptors exist on the glutamatergic axons/terminals which make synapses on the VLPO neurons. By enhancing Cl^- efflux from these axons, GABAergic agents such as propofol depolarizes these terminals and stimulates the release of glutamate, which increases the activity of VLPO neurons, and thus potentiates GABAergic inhibition of arousal systems [12]. Clearly, this mechanism could be a significant component of the sedative action of GABA and GABAergic agents. In contrast, glycine stimulation didn't produce any significant effect, suggesting that glycine receptor is absent in the VLPO. Glycine is also a major inhibitory neurotransmitter in the central nervous system. The finding in the current study is consistent with common understanding that glycinergic neurotransmission mostly occurs at the levels of spinal cord and brainstem [13].

The results of this experiment further confirmed the mediatory role of VLPO in sedation induction, and more specifically, the effect is likely mediated through the GABA receptors in the VLPO neurons.

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