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Research report

Activation of $GABA_A$ and $GABA_B$ receptors in the lateral septum increases sucrose intake by differential stimulation of sucrose licking activity

Arojit Mitra, Christophe Lenglos, Elena Timofeeva *

Faculté de Médecine, Département de Psychiatrie et de Neurosciences, Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Université Laval, Québec, QC G1V 4G5, Canada

HIGHLIGHTS

- Injection of muscimol and baclofen into the lateral septum increased sucrose intake.
- Muscimol but not baclofen decreased the latency to initiate sucrose licking.
- Baclofen but not muscimol increased the total licking time.

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ABSTRACT

The present study was aimed to determine how direct injections into the lateral septum (LS) of muscimol and baclofen, $GABA_A$ and $GABA_B$ receptor agonists, respectively, affect intake of 10% sucrose and sucrose licking activity in rats. The effects of muscimol and baclofen on the 1-h intake of sucrose and sucrose licking activity were tested at low (350 pmol), medium (876 pmol), and high (1752 pmol) doses. The medium and high doses of muscimol and the high dose of baclofen significantly increased 1-h sucrose intake. The total sucrose lick number was significantly increased by the medium dose of muscimol and the high dose of baclofen. An increase in sucrose licking activity induced by muscimol but not baclofen occurred in the first 15 min after injections. The medium and high doses of muscimol but not baclofen significantly decreased latency to initiate the first lick of sucrose. The total licking time calculated as the sum of the duration of all sucrose lick clusters showed a significant increase by the high dose of baclofen but not by any dose of muscimol. Therefore, the $GABA_A$ and $GABA_B$ LS mechanisms appear to be involved in stimulating sucrose intake, but this stimulation occurs by differential regulation of the sucrose licking activity. Muscimol intra-LS administration led to a short-latency rapid increase in sucrose licking. In contrast, baclofen did not decrease latency to initiate licking, but significantly increased total licking duration.

1. Introduction

The lateral septum (LS) is a forebrain subcortical structure that largely innervates the anterior, lateral, dorsomedial, and posterior hypothalamus [1–3]. The LS is mainly composed of projecting small- and medium-size neurons that produce the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) [4–6]. The LS neurons are regulated by GABAergic neurotransmission as suggested by the high density of the GABA-positive presynaptic boutons that make symmetric synapses on the soma and dendrites of the LS neurons [7]. The GABAergic input to the LS neurons may be produced by recurrent axonal collateral of the LS neurons [7–10] and by the GABAergic input to the LS from other GABAergic sources such as from the GABAergic neurons that project from the nucleus

Abbreviations: AcbSh, nucleus accumbens shell; cc, corpus callosum; CRF_2-R, type 2 receptor of corticotropin releasing factor; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; $GABA_A$, type A receptor of gamma-aminobutyric acid; $GABA_B$, type B receptor of gamma-aminobutyric acid; HPA, hypothalamic pituitary adrenal axis; ICI, inter-cluster intervals; LH, lateral hypothalamus; LS, lateral septum; LSd, dorsal part of the lateral septum; LSi, intermediate part of the lateral septum; LSv, ventral part of the lateral septum; MS, medial septum; SHi, septohippocampal nucleus.

* Corresponding author at: Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Y3150, 2725 Chemin Sainte-Foy, Québec, QC G1V 4G5, Canada. Tel.: +1 418 656 8711x3749; fax: +1 418 656 4942.
E-mail addresses: Arojit.Mitra@criucpq.ulaval.ca (A. Mitra), Christophe.Lenglos@criucpq.ulaval.ca (C. Lenglos), Elena.Timofeeva@fmed.ulaval.ca (E. Timofeeva).

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accumbens to the LS [11]. GABA affects the neuronal activity *via* the $GABA_A$ and $GABA_B$ receptors. Activation of $GABA_A$ and $GABA_B$ receptors by their agonists facilitates entry of chloride ions or exit of potassium ions, respectively, that typically hyperpolarizes the neuron producing a decrease in neuronal activity [12–15]. The subunits of the $GABA_A$ and $GABA_B$ receptors are expressed in the LS [16–20].

There is strong evidence that the LS can modulate the activity of the hypothalamic neurons and the physiological functions directly regulated by the hypothalamus [6,21]. Activation of the subset of the LS neurons expressing the type 2 receptor of corticotropin-releasing factor (CRF2-R) affects the levels of activity of the hypothalamic-pituitary adrenal axis (HPA) [22]. In addition, direct activation of the CRF2-R by specific agonists injected directly into the LS significantly decreased food intake in rats [23,24]. In contrast, overeating of 10% sucrose developed by submitting rats to repeated cycles of food restriction was associated with a decrease in the LS levels of CRF2-R and lower stress-induced activation of the LS neurons [25]. Therefore, activity of the LS neurons is related to feeding behavior.

An early study that implicated bilateral electrolytic lesions of the LS showed a significant increase in the number of licks of sucrose but not water [26]. This increase in sucrose-licking activity in the LS-lesioned rats was independent of thirsting or fasting and was not eliminated by sucrose preloading [26]. Although the LS lesions clearly increased sucrose intake, this technical approach did not explain whether this effect depends on a decrease in LS neuronal activity, on an increase in LS neuronal inhibition or on the ablation of the fibers that run to the LS from the hippocampus, hypothalamus, and brainstem [27–29]. These difficulties in interpreting studies involving the electrolytic ablations of the LS were also encountered in the contradictory effects of LS lesions on food intake and body weight gain [30–36]. Our recent study that showed a decrease in stress-induced induction of the expression of c-*fos* mRNA in the LS neurons in sucrose-overeating rats [25] did not explain if this effect was due to a decrease in activation or an increase in inhibition in the LS neurons.

The present study was designed to assess how direct LS inhibition affects the intake of 10% sucrose in rats. We used intra-LS injections of $GABA_A$ specific agonist muscimol or $GABA_B$ specific agonist baclofen to dissociate the role of $GABA_A$ and $GABA_B$ mechanisms modulating the intake of sucrose and sucrose licking activity. The results showed that muscimol and baclofen increased sucrose intake. However, this increase depended on differential changes in sucrose licking activity.

2. Materials and methods

2.1. Animals

Male ($n = 16$) Sprague Dawley rats (Canadian Breeding Laboratories, St-Constant, QC, Canada) with a weight range of 300–350 g at the time of surgery were housed in the same room in individual plastic cages and maintained in an environment with controlled temperature ($23 \pm 1\,^{\circ}C$) and light (12 h:12 h light–dark cycle, light on between 06:00 and 18:00 h) with *ad libitum* access to chow and tap water. All rats were cared for and handled according to the Canadian Guide for the Care and Use of Laboratory Animals, and the present protocol was approved by our institutional animal care committee.

2.2. Surgery and acclimatization

Surgery procedures were performed under isoflurane (3% in oxygen) anesthesia along with systemic (bubrenorphine, 0.02 mg/kg, i.p.) and local [mixture of lidocaine (7 mg/kg) and bupivacaine (3.5 mg/kg), s.c.] analgesics. Bilateral 26-gauge stainless steel cannulas (Plastics One, Roanoke, VA) were implanted using the stereotaxic technique. The guide cannulas were aimed at the LS using the following coordinates: 1 mm rostral to the bregma, 0.8 lateral to the midline, and 5 mm ventral to the skull surface [37]. The guide cannulas were fixed to the skull using 3–4 stainless steel screws and dental cement. Fitting stainless steel obturators were inserted inside each cannula to prevent blockage and infection. Each rat received subcutaneous injection of anti-inflammatory meloxicam (1 mg/kg) for 3 days after surgery and recovered for 7 days before the behavioral experiments began.

2.3. Drugs and intra-LS microinjections

Muscimol and baclofen were purchased from Tocris Bioscience (Bristol, UK). Both drugs were dissolved in sterile artificial cerebrospinal fluid (CSF; Harvard Apparatus, Holliston, MA). The same CSF was injected as vehicle (0 pmol). Rats were habituated for 2–3 days to the operant chamber (Med Associates, St. Albans, VT) and manipulations for intra-nucleus injections by mock injection sessions with the removal and insertion of steel obturators followed by placement in operant cages with *ad libitum* access to water. All rats were given 2 days of 1 h access to 10% sucrose solution and tap water in the operant cage to counter taste neophobia.

Rats were gently restrained, and a 33-gauge stainless steel injection cannula was inserted bilaterally into a guide cannula. The injection cannula extended 0.6 mm beyond the distal tip of the guide cannula. Each rat received simultaneous bilateral infusion of 0.5 μl of either CSF or drug at a flow rate of 0.25 μl/min. The injectors were left in place after the infusions for an additional 2 min to ensure drug diffusion and prevent drug leakage at the dorsal sites. The muscimol rat group ($n = 8$) received bilateral injections of 0, 350, 876, and 1752 pmol of muscimol. The baclofen rat group ($n = 8$) received injections of equimolar doses of baclofen. Injections were performed in counterbalanced order and were separated by at least 48 h. Immediately after the injections, the rats were placed in operant chambers with a pre-weighed quantity of 10% sucrose solution and water in bottles equipped with optical lickometers to collect time stamps of licking events during the 1-h session. Licking time stamps were acquired using a multi-channel acquisition system (Tucker-Davis Technologies, Alachua, FL). Sucrose and water consumption during the 1-h session was measured by weighing the drinking bottles before and after the session. The time taken for the first lick to occur after the rat was placed in the operant cage was taken as a measure of lick latency.

2.4. Licking microstructure

The number of infrared beam interruptions by tongue protrusion to bottle spout was assessed as the number of licks performed by rats during 1 h after LS bilateral injection of CSF or GABA agonists. In addition to the total 1-h lick number, the sucrose lick number was estimated in each 15-min epoch following injections (within 0–15, 15–30, 30–45, and 45–60 min). Sucrose licking clusters were determined as high-frequency (6–9 Hz) licks occurring in a run of three or more licks interrupted by pauses or inter-cluster intervals (ICI) of 500 ms or longer. This timing criterion was based on the Davis and Perez analyses of lick clustering that integrates more than 90% of all recorded licks [38]. A custom MATLAB program (R2010a, The MathWorks™) was used to sort the lick data into lick clusters based on ICI cutoffs of ≥500 ms. For each experimental condition, we calculated the number of clusters and cluster duration (in second). The total licking time was calculated as the sum of the duration of all clusters during 1-h access to sucrose. The inter lick intervals (ILI) within the clusters were discriminated between 60 and 500 ms ($60\text{ ms} \leq \text{ILI} < 500\text{ ms}$). This time window was taken