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Research report

Cortical arousal induced by microinjection of orexins into the paraventricular nucleus of the rat

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Abstract

Orexin-A is a neuropeptide which has been suggested to be involved in sleep and arousal mechanisms. Orexin-A, for example, stimulates arousal when administrated intracerebroventricularly to rats. We attempted to identify specific neural sites of orexin-A and orexin-B action. Orexin-A and orexin-B were microinjected into the medial parvocellular subdivision of the paraventricular nucleus (PVN) in anesthetized, spontaneously breathing rats, and cortical arousal and yawning responses were assessed. Cortical arousal responses were monitored with the electrocorticogram (ECoG), and yawning responses were evaluated by monitoring intercostal electromyograms as an index of inspiratory activity and digastric electromyograms as an indicator of mouth opening. We also measured blood pressure and heart rate during yawning responses, since yawning is accompanied by changes in autonomic activity. Microinjection of orexin-A into the PVN elicited an arousal shift in the ECoG to lower voltage and faster rhythms. This cortical arousal response was followed by a single large inspiration with mouth opening, i.e. a yawning response. On the other hand, microinjection of orexin-B into the PVN elicited an arousal shift in the ECoG without yawning responses. These results demonstrate that an orexin receptive site for triggering arousal/yawning responses exists in the PVN, and suggest that the PVN is involved in arousal mechanisms.

Keywords: Orexin; Arousal; Paraventricular nucleus; Yawning; Electrocorticogram; Rat

1. Introduction

We recently reported that a stereotyped yawning response can be evoked by microinjection of L-glutamate, cyanide and a nitric oxide-releasing compound (NOC12) into the medial parvocellular subdivision of the paraventricular nucleus (PVN) in anesthetized, spontaneously breathing rats [13,23]. In those studies, we recorded the electrocorticogram (ECoG) to evaluate arousal responses during yawning, and found that ECoG arousal, represented by lower voltage and faster rhythms, occurred before the final yawning behavior during both spontaneous yawning and yawning responses evoked by chemical stimulation. This arousal effect was of considerable significance because the data were obtained under anesthesia. Although the PVN is essential for the occurrence of yawning [1], our previous studies raised the possibility that the PVN may play an important role in triggering arousal mechanisms. We therefore suggested that the yawning model is appropriate for investigating arousal mechanisms in rats.

Orexins (A and B) are a recently identified family of neuropeptides originally believed to be important mediators of food intake [22]. Apart from appetite regulation, orexins have now emerged as important regulators of sleep and arousal [3,8,21,25]. For example, Chernelli et al. [3] proposed that orexin knockout mice may be a model for human narcolepsy. They further observed that orexin neurons innervate neurons critical for controlling arousal in the ascending cortical activation system. Hagan et al. [8] demonstrated that orexin-A activates locus coeruleus cell firing in vitro and in-

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creases arousal in rats in vivo. Moreover, Piper et al. [21] reported that orexin-A modifies the sleep–wake cycle of rats. In those studies, orexin-A stimulated arousal after intracerebroventricular injection, but the precise site of action within the brain for orexin-A to stimulate arousal remains unknown.

We sought to determine whether the PVN is orexin sensitive by microinjecting orexin-A into the specific region within the PVN where a stereotyped yawning response was induced by L-glutamate. The effect of orexin-B microinjection into the PVN was also examined. The doses of orexin-A and orexin-B used in the present study were at least 1000 times smaller than those used for feeding responses in microinjection studies of the PVN [7,26]. All microinjections were done with multi-barrel glass micropipettes which provide especially high resolution, thus stimulating only a restricted region within the PVN.

2. Materials and methods

Experiments were performed on 12 male Wistar rats weighing 300–450 g. The rats were anesthetized with 50 mg/kg pentobarbital sodium intraperitoneally, and additional doses were given as needed. All experiments were approved by the Animal Experimentation Ethics Committee of the Toho University School of Medicine. All efforts were made to minimize animal suffering and the number of animals used. Surgical procedures were essentially the same as described previously [23]. In brief, catheters were placed in the femoral artery to monitor arterial blood pressure (BP). Heart rate (HR) was measured from the BP pulse with a tachometer (AT-601G, Nihon Kohden, Japan). To monitor respiratory activity, a pair of twisted wire electrodes, insulated except for 1 mm at the tips, were implanted into the lower intercostal space, by way of a 23-gage hypoder-

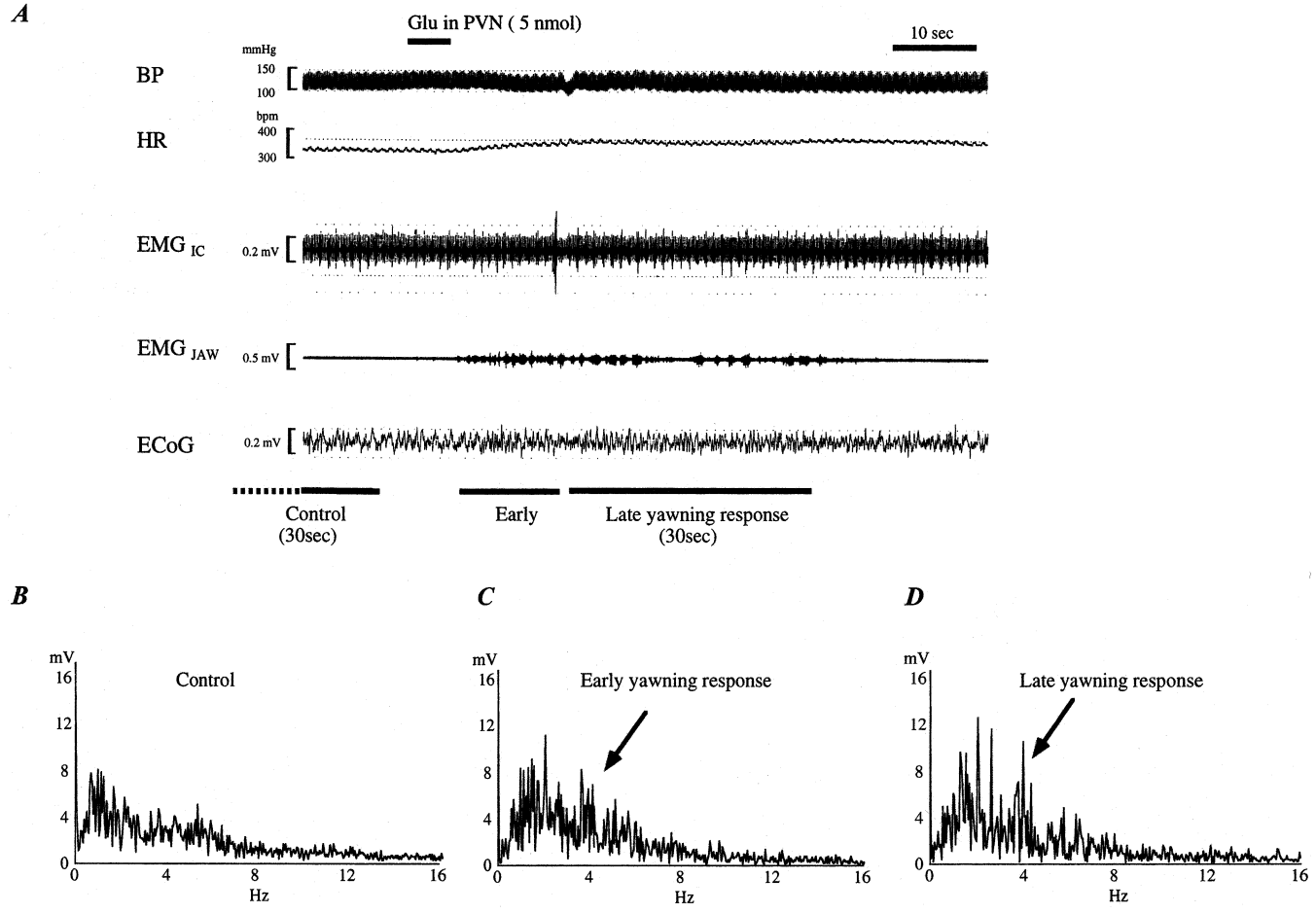

Fig. 1. Representative yawning responses to injection of L-glutamate (5 nmol) in the PVN (A). Changes in the ECoG recording before (B), during (C) and after (C) yawning are shown in the lower panel. During the control period before chemical stimulation, the ECoG of anesthetized rats was characterized by very slow waves (< 2 Hz). Note that the waves shifted to lower amplitude faster rhythms (indicated by arrows) after microinjection of L-glutamate into the PVN. BP, blood pressure; HR, heart rate; EMG_{IC}, intercostal electromyogram; EMG_{JAW}, digastric electromyogram; ECoG, cortical electroencephalograph.

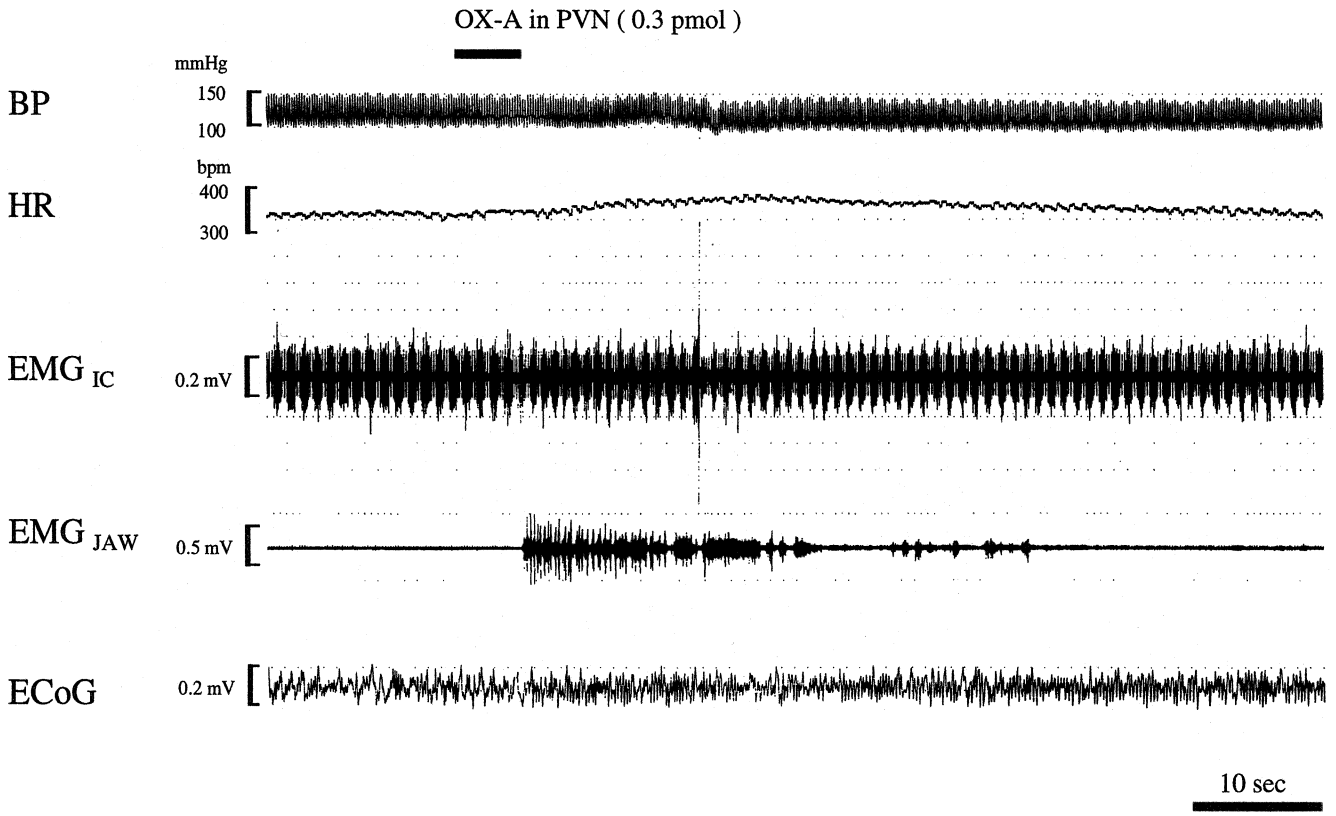

Fig. 2. Representative yawning/arousal responses evoked by microinjection of orexin-A (0.3 pmol) into the PVN. Note that the ECoG waves shifted to lower voltages after microinjection of orexin-A into the PVN. Abbreviations as in Fig. 1.

mic needle; the needle was then withdrawn, leaving the wires in the intercostal muscle. Similarly, pairs of wire electrodes were implanted in the digastric muscle to monitor mouth opening activity. For ECoG recordings, holes were drilled in the skull and two screw electrodes were implanted. ECoG signals were amplified and filtered (0.1–50.0 Hz bandpass). These polygraphic signals were all stored in a DAT data recorder (PC208Ax, Sony, Japan) for further analysis. Rectal temperature was maintained at 37°C with a heating lamp.

The animals were fixed prone in a stereotaxic frame and a parietal craniotomy was performed. For drug microinjections into the PVN of the hypothalamus, we constructed a four-barrel glass micropipette which was connected to a fine cannula. The free end of the cannula was attached to a picopump (PV830 Pneumatic, WPI) for injections. The volumes injected were monitored under a dissecting microscope and measured from the movement of the fluid meniscus. The barrels were filled with either 0.1 M L-glutamate, 10–100 μg/ml orexin-A (Phoenix Pharmaceuticals, INC), 10–100 μg/ml orexin-B (Phoenix Pharmaceuticals, INC), or artificial cerebrospinal fluid (ACSF: 147 mM NaCl, 3 mM $NaHCO_3$, 3.5 mM KCl, 1.3 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.5 mM NaH_2PO_4 and 1.1 mM urea; pH 7.30–7.35). L-Glutamate, orexin-A and orexin-B were freshly dissolved in ACSF, respectively. As a control for L-glutamate, orexin-A and orexin-B, the same amount of ACSF was injected in responsive sites (three rats). The chemicals were obtained from Wako, Japan. The dura overlying the cortex was opened for advancement of the micropipette, which was inserted into an area 0.2–0.3 mm lateral to the midline and 1.1–2.4 mm posterior to the bregma, 5.8–7.2 mm vertical from the parietal dura, according to the Paxinos and Watson [20] atlas of the rat brain. We made a systematic search for the sites from which yawning responses were evoked by microinjection of L-glutamate (10–100 nl). After exploratory tracking with L-glutamate, we identified sites where maximal yawning responses were obtained. A small volume (10–100 nl) of either orexin-A or orexin-B solution was then slowly injected into the same responsive site to evaluate whether a yawning response could be induced. On completion of the experiment, successful stimulation sites were marked by 0.5 M acetate-pontamine for histological examination. The rat was then deeply anesthetized with pentobarbital sodium (100 mg/kg) to remove the brain.

After the experiments, ECoG signals were displayed on an oscilloscope, together with intercostal EMG. For power spectrum analysis, the ECoG signal was digitized at a sampling rate of 1024 Hz and subjected to fast Fourier transforms. Analyses were performed on data sampled during pre-injection (control), early yawning

and late yawning (recovery) periods of each response. The early yawning period was selected on the basis of visual inspection as the early phase of a yawning response, corresponding to an initial depressor response prior to a final yawning event (a single large inspiration). The late yawning (recovery) period was 15–120 s until ECoG activity returned to pre-injection activity levels. In the case of orexin-B, analyses were performed on data sampled during pre-injection (control), and post-injection of each response.

Statistical analyses were carried out by one-way analysis of variance, followed by Sheffe's test to correct for multiple comparisons of treatments. A probability value of 0.05 was adopted as the level of significance.

3. Results

We first made a systematic search for yawning-responsive sites in and around the PVN by microinjection of L-glutamate. Microinjection of L-glutamate in the medial parvocellular subdivision of the PVN elicited a stereotyped yawning response. We confirmed that no other sites in and around the PVN could elicit this stereotyped response. A typical example of such a stereotyped yawning response induced by L-glutamate (5 nmol) is shown in Fig. 1A. The response pattern was characterized by an initial depressor phase followed by a final yawning event (a single large inspiratory effort). Power spectral analysis of ECoG waves is shown in the lower panel. During the control period before chemical stimulation, the ECoG of anesthetized rats was characterized by high voltage and very slow waves (< 2 Hz, Fig. 1B). Concurrent with the depressor response, ECoG waves shifted to lower voltage and faster rhythms (~ 4 Hz, Fig. 1C). The component of faster rhythms remained after the final yawning behavior (Fig. 1D), and returned gradually to control levels. These results were comparable to those of our previous studies [13,23].

We next examined the effect of microinjection of orexin-A in the specific region with the stereotyped yawning. Fig. 2 shows a typical example of the effect of orexin-A injection into the site where the stereotyped yawning response was elicited by L-glutamate injection. Orexin-A injection (0.3 pmol) qualitatively caused similar sequential events of a stereotyped yawning response. The initial response represented an increase in HR and

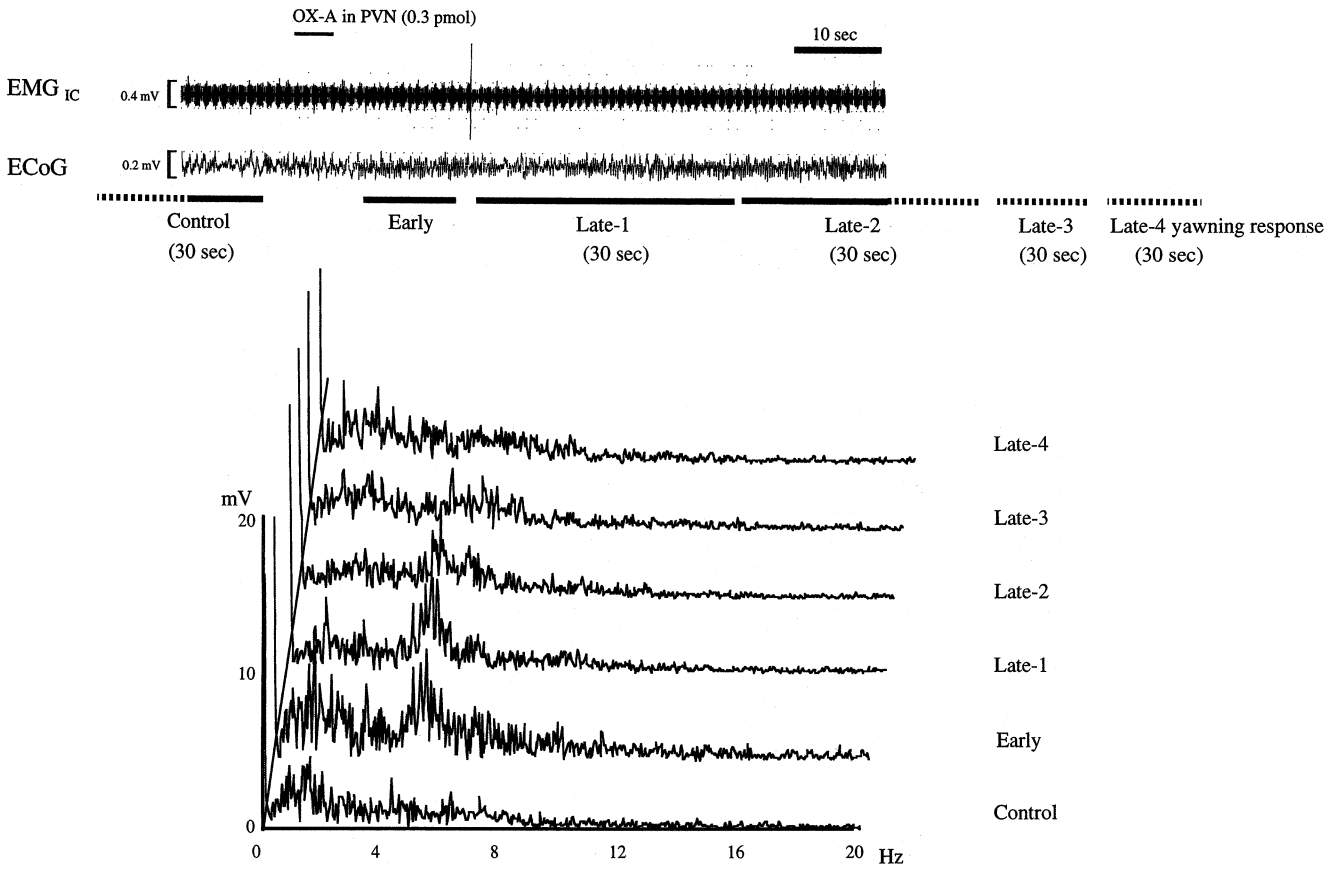

Fig. 3. Typical changes in the cortical electroencephalograph recording during yawning induced after microinjection of orexin-A (0.3 pmol) into the PVN. The very slow waves (< 2 Hz) during the control period before chemical stimulation were replaced by faster rhythms (~ 6 Hz) immediately before yawning. Abbreviations as in Fig. 1.

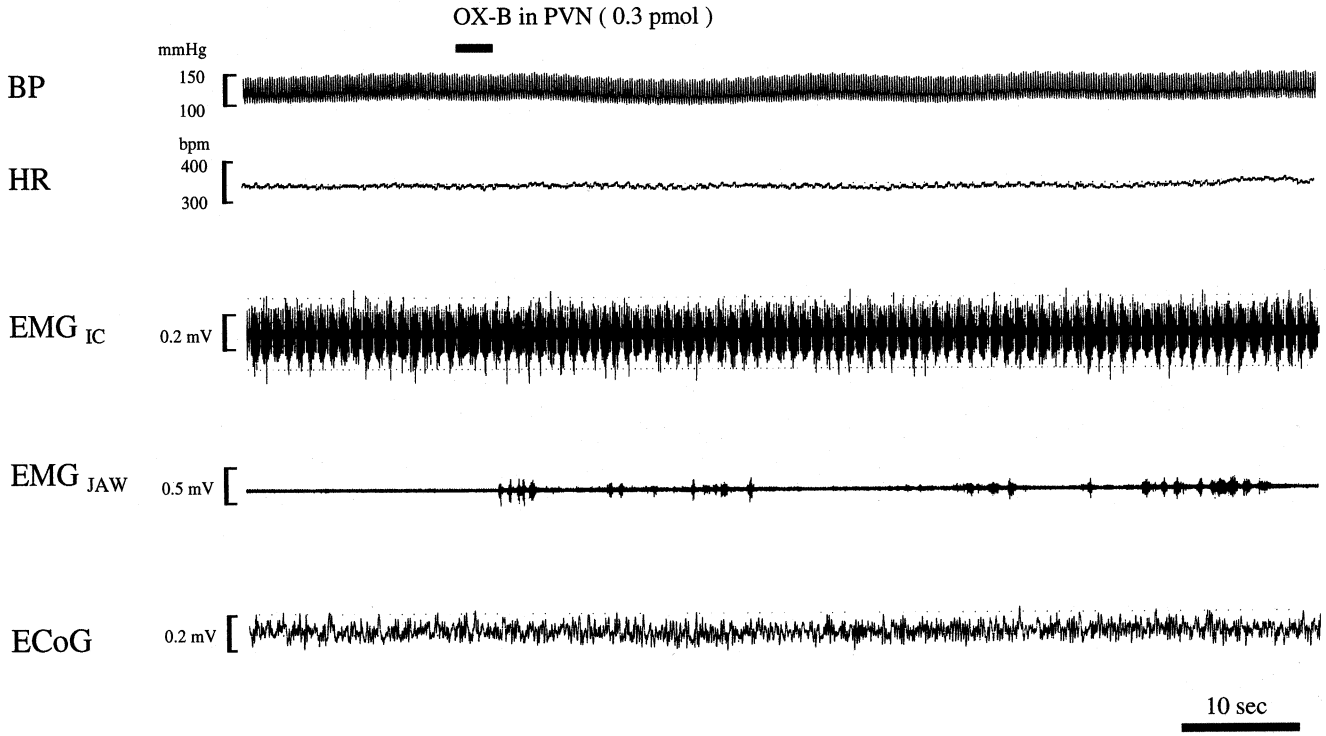

Fig. 4. Representative response evoked by microinjection of orexin-B (0.3 pmol) into the PVN. No yawning responses emerged after microinjection of orexin-B into the PVN. Abbreviations as in Fig. 1.

an arousal shift in the ECoG to lower voltage, as described precisely in the next paragraph. EMG_{JAW} showed quite rhythmical activity. These early yawning responses were followed by the development of a single large inspiration. After the final yawning behavior, BP and HR returned gradually to control levels. As for ECoG, the component of lower voltage remained after the final yawning behavior, but this also returned gradually to control levels.

Power spectral analysis of ECoG waves revealed that an early change in ECoG occurred prior to a yawn, as shown in Fig. 3. The high voltage and very slow waves during the control period before chemical stimulation (<2 Hz, Fig. 3: Control), were replaced by faster rhythms concurrent with the cardiovascular and EMG_{JAW} responses (~ 6 Hz, Fig. 3: Early). The component of faster rhythms remained after the final yawning behavior (Fig. 3: Late-1, Late-2, Late-3), and returned gradually to control levels (Fig. 3: Late-4). We examined the effects of orexin-A injection in 12 sites (one site per rat) and found that orexin-A injection caused the yawning/arousal response in all sites examined.

We further evaluated the effect of orexin-B injection in the specific regions where the stereotyped yawning responses were elicited by both L-glutamate and orexin-A injections, as shown a typical example in Fig. 4. In contrast to results obtained by orexin-A, no major effects of orexin-B were observed at any dose; orexin-B (0.3 pmol) did not induce the yawning response, the increase in HR was weak or negligible, and EMG_{JAW} showed a little activity. However, power spectral analysis of ECoG waves revealed that a change in ECoG occurred after administration, as shown in Fig. 5. During the control period before chemical stimulation, the ECoG of anesthetized rats was characterized by high voltage and very slow waves (<2 Hz, Fig. 5: Control). ECoG waves shifted to faster rhythms (~ 6 Hz) after the injection (Fig. 5: Post-1). This arousal shift in ECoG reached as maximum 30–60 s after the injection (Fig. 5: Post-2). The component of faster rhythms remained for another 150 s (Fig. 5: Post-3, Post-4, Post-5), and returned gradually to control levels (Fig. 5: Post-6). Similar arousal responses to orexin-B injection were obtained from all sites examined (five sites from five rats).

Light microscopic examination revealed that both orexin-A and orexin-B responsive sites were located in the medial part of the rostral PVN. No responsive sites for yawning/arousal response were identified in the lateral part of the rostral PVN, in the caudal PVN, or regions around the PVN.

4. Discussion

This is the first study showing effects of microinjecting orexin-A and orexin-B into the PVN on arousal

parameters recorded in anesthetized rats. Microinjection of orexin-A into the PVN induced yawning, together with an arousal shift in the ECoG. On the other hand, orexin-B induced an arousal shift in the ECoG without a yawning response. These findings suggest that the PVN is an orexin-sensitive site, and that this site might be involved in an arousal signaling pathway.

Our observation that the PVN is an orexin-sensitive site is supported by several lines of evidence. Trivedi et al. [27] reported that orexin receptors are found in the PVN, and Date et al. [5] provided other evidence showing that orexin fibers are extensively found at that site. Further evidence comes from Dube et al.'s [7] observation that orexin-A enhances food intake when injected into the PVN. These data together raise the possibility that the PVN may be one of the important targets receiving orexinergic inputs from the posterior and lateral hypothalamus.

The receptor expressed in the PVN might be the orexin receptor 2 (OX_2R). Two receptor types have been identified for orexin-A and orexin-B: orexin receptor 1 (OX_1R) and OX_2R [27]. Whereas OX_2R binds both orexin-A and orexin-B at high affinity, OX_1R binds orexin-A preferentially over orexin-B [12]. Our data showing that both orexin-A and orexin-B induced arousal shifts in the ECoG suggest that this effect is mediated by OX_2R. The suggestion is supported by data of Trivedi et al. [27] who found that OX_2R is predominantly found in the PVN. However, the lack of orexin-B to induce either yawning or cardiovascular responses raises another possibility that the respiratory and cardiovascular effects induced by orexin-A might be OX_1R mediated.

Neurons responsible for the effects induced by either orexin-A or orexin-B might be the parvocellular neurons of the PVN. This suggestion was based on our earlier finding showing that the parvocellular division in the PVN is essential for the stereotyped yawning response, involving the arousal shift in the ECoG [13]. This is consistent with the observation of Date et al. [5] who found that orexin fibers project to the parvocellular division of the PVN. In this connection, intercerebroventricular administration of orexin-A induced c-fos mRNA in the parvocellular division of the PVN [15]. Consistently, corticotropin-releasing factor, which is mainly synthesized by the parvocellular neurons in the PVN, is increased by intercerebroventricular administration of orexin-A [8,11,27].

We previously reported that neurons responsible for yawning might be the oxytocinergic parvocellular neu-

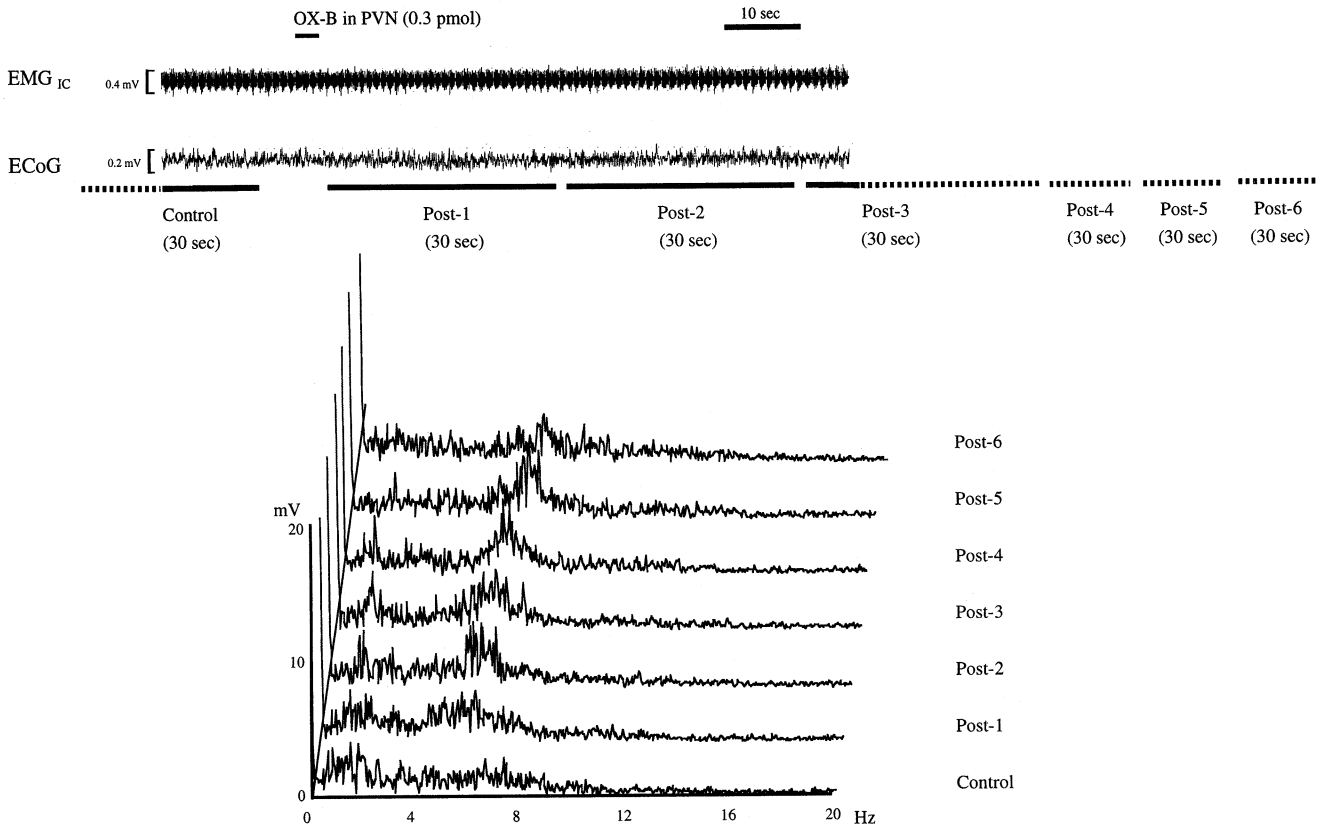

Fig. 5. Typical changes in the cortical electroencephalograph recording during yawning induced after microinjection of orexin-B (0.3 pmol) into the PVN. During the control period before chemical stimulation, the ECoG of anesthetized rats was characterized by very slow waves (< 2 Hz). The waves shifted to faster rhythms (~ 6 Hz) after microinjection of orexin-B into the PVN. Abbreviations as in Fig. 1.

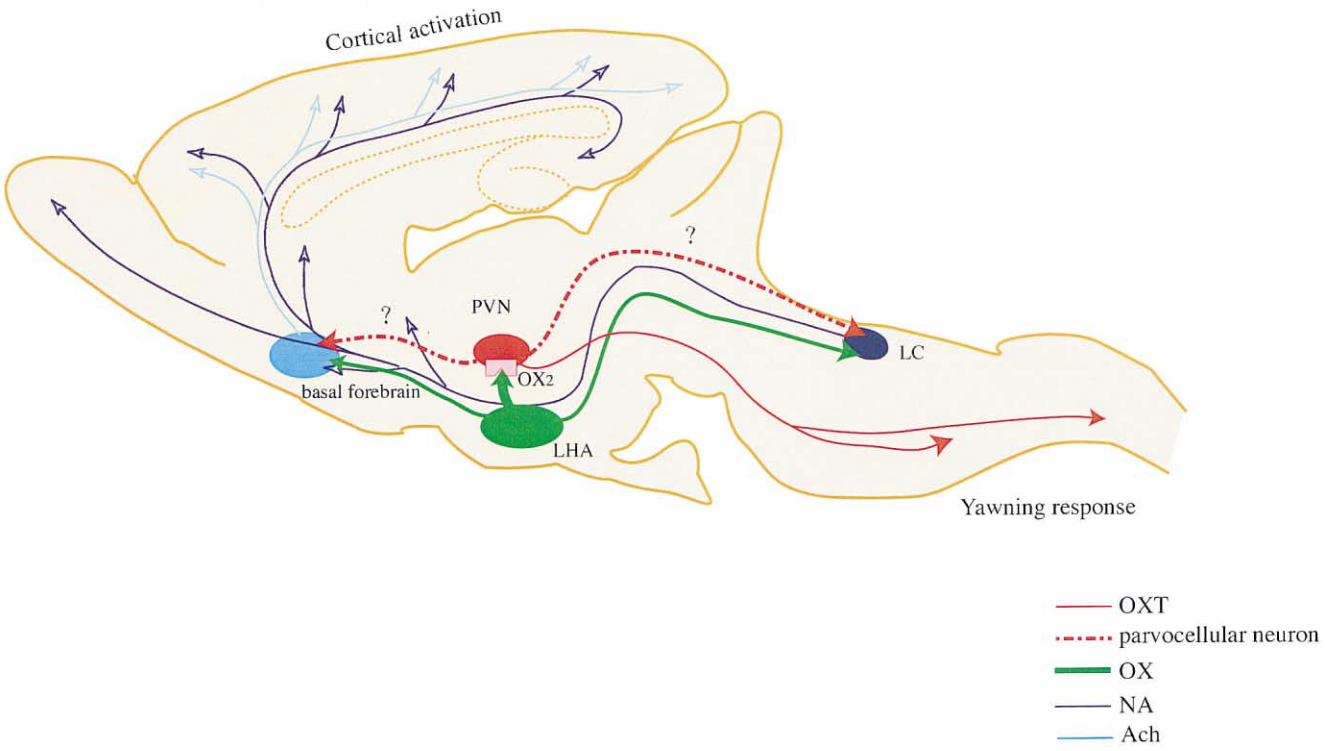

Fig. 6. Schematic representation of our model on yawning/arousal responses mediated through the PVN. Orexin neurons from the LHA may directly activate neurons in the PVN, which in turn project to the LC or the basal forebrain. Oxytocinergic neurons from the PVN project to the lower brainstem, including the facial nucleus and respiratory related neurons which are implicated in the yawning response. OXT, oxytocin; OX, orexin; NA, noradrenaline; Ach, acetylcholine.

rons in the PVN projecting to the lower brain stem [23]. Our suggestion was principally based on the report of Sawchenko and Swanson [24] who demonstrated that oxytocinergic parvocellular neurons in the PVN send descending axons to the lower brain stem, a region involved in arousal, respiratory, cardiovascular, and other autonomic functions. We therefore further suggest the possibility that at least orexin-A activates the oxytocinergic parvocellular neurons in the PVN projecting to the lower brain stem. This possibility should be investigated further through the use of immunohistochemical techniques.

Recent studies show that dense arborization of orexin axons is detected in the locus coeruleus (LC), a region involved in arousal mechanisms [10]. Orexin axons also innervate cholinergic neurons of the basal forebrain [12], i.e. another region implicated in sleep/arousal regulation [17]. The source of orexin neurons in either LC or the basal forebrain is suggested to be the lateral hypothalamic area (LHA) [10,12]. Although orexin neurons in either LC or the basal forebrain may directly extend from the LHA, our present data suggest another signaling pathway of the arousal mechanism via the PVN. Namely, orexin neurons from the LHA may directly activate parvocellular neurons in the PVN, which in turn project to the LC or the basal forebrain (Fig. 6).

Much of the focus on orexin peptides has been related to the ability of orexin-A to stimulate food intake or arousal responses when intracerebroventricularly administrated [7,8,21,26]. However, the effect of orexin-B has been problematic in most studies. The present findings that orexin-B preferentially stimulated the arousal shift in the ECoG led us to propose that this substance may have a role in arousal regulation, whereas orexin-A may function in various autonomic responses, including the arousal response. This view is consistent with the data of Horvath et al. [10] who reported that orexin-B enhances activity of the LC noradrenergic system which is important in arousal regulation. The difference in function of orexin-A and orexin-B is at this time unknown, and remains to be clearly demonstrated.

It may be argued that injections of orexins into the PVN were large, and could have diffused into the LHA, which has many of the same projections as the PVN, and could account for current findings. This possibility is not likely, because all microinjections were performed with glass micropipettes in the present study, thus restricting stimulation to portions within the PVN. Histological examination revealed that injections diffused only within the medial parvocellular subdivision of the PVN. We also confirmed that no other area in and around the PVN elicited the arousal/yawning re-

sponse. As determined by onset latencies of arousal/yawning responses, neurons in the PVN responded to orexins within 30 s and returned to near baseline levels within 3 min, whereas the feeding [7,26], drinking [14] or arousal responses [21] in other studies occurred first after 1 or 2 h, and the effects lasted more than several hours.

The yawning response has important physiological significance for arousal in anesthetized animals. It is apparent from observing human subjects that yawning is not a behavior restricted to mouth opening, but is a coordinated motor pattern characterized by a deep inspiration and stretching of the trunk [2]. Yawning is also accompanied by changes in autonomic function, including a depressor response [23], lacrimation and erection [9,18]. Furthermore, yawning is a phenomenon that subserves arousal [4]. To evaluate these various physiological aspects of yawning, we monitored polygraphic measures representing a yawning response in anesthetized, spontaneously breathing rats. It is important to emphasize that if we used conscious animals, the data obtained would be almost certainly restricted to counting the number of mouth openings, which shows only one aspect of the entire yawning response.

Whereas the ECoG of anesthetized rats is normally characterized by high voltage and very slow waves (< 2 Hz), faster rhythms (~ 4–6 Hz) became dominant after chemical stimulation of the PVN. Since this arousal shift in the ECoG was usually accompanied by yawning responses, we suggest that the PVN mediates the cortical activation related to yawning. In this respect, Dringenberg and Vanderwolf [6] demonstrated that several pathways contribute to ECoG activation as well as arousal mechanisms. Our findings indicate that the PVN plays a significant role in the arousal mechanism related to yawning.

Narcolepsy, a disorder characterized by excessive daytime sleepiness, cataplexy, and striking transitions from wakefulness into rapid eye movement sleep, is apparently caused by orexin and orexin receptor deficiency in human [19] and animal models [3,16]. The type of orexin receptor deficiency in this disorder is OX_2R, which is predominantly found in the PVN [16]. These data together suggest orexin as a major neurotransmitter involved in sleep and arousal mechanisms, and further strengthen our hypothesis that the PVN plays an important role in arousal systems.

In conclusion, microinjection of orexin-A into the PVN elicited an arousal/yawning response, whereas orexin-B injection induced the arousal response alone. The results demonstrate that an orexin receptive site for triggering arousal/yawning responses exists in the PVN. Our finding should open a new avenue of research to investigate the role of the PVN in arousal regulation.

References

[1] Argiolas A, Melis MR, Mauri A, Gessa GL. Paraventricular nucleus lesion prevents yawning and penile erection induced by apomorphine and oxytocin but not by ACTH in rats. Brain Res 1987;421:349–52.

[2] Bertoline A, Gessa GL. Behavioural effects of ACTH and MSH peptides. J Endocrinol Invest 1981;4:241.

[3] Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999;98:437–51.

[4] Concu A, Ferrari W, Gessa GL, Mereu GP, Tagliamonte A. EEG changes induced by the intraventricular injection of ACTH in cats. In: Levin P, Koella WP, editors. 2nd Europ. Cong. Sleep Res. Rome, 1974. p. 321–325.

[5] Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nagazato M. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc Natl Acad Sci USA 1999;96:748–53.

[6] Dringenberg HC, Vanderwolf CH. Involvement of direct and indirect pathways in electrocorticographic activation. Neurosci Biobehav Rev 1998;22:243–57.

[7] Dube MG, Kalra SP, Kalra PS. Food intake elicited by central administration of orexins/hypocretins: identification of hypothathalamic sites of action. Brain Res 1999;842:473–7.

[8] Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DNC, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci USA 1999;96:10911–6.

[9] Heusner AP. Yawning and associated phenomena. Physiol Rev 1946;26:156–68.

[10] Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, Van Den Pol AN. Hypocretin (Orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J Comp Neurol 1999;415:145–59.

[11] Iida T, Nakahara K, Murakami T, Hanada R, Nakazato M, Murakami N. Possible involvement of orexin in the stress reaction in rats. Biochem Biophys Res Commun 2000;270:318–23.

[12] Kilduff TS, Peyron C. The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci 2000;23:359–65.

[13] Kita I, Sato-Suzuki I, Oguri M, Arita H. Yawning responses induced by local hypoxia in the paraventricular nucleus of the rat. Behav Brain Res 2000;117:119–26.

[14] Kunii K, Yamanaka A, Nambu T, Matsuzaki I, Goto K, Sakurai T. Orexins/hypocretins regulate drinking behaviour. Brain Res 1999;842:256–61.

[15] Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, Yamashita H. Centrally administered orexin/hypocretin activates HPA axis in rats. NeuroReport 2000;11:1977–80.

[16] Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98:365–76.

[17] Manfridi A, Brambilla D, Mancia M. Stimulation of NMDA and AMPA receptors in the rat nucleus basalis of meynert affects sleep. Am J Physiol 1999;277:R1488–92.

[18] Melis MR, Argiolas A, Gessa GL. Oxytocin-induced penile erection and yawning: site of action in the brain. Brain Res 1986;398:259–65.

[19] Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000;355:39–40.
[20] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 2nd ed. San Diego, CA: Academic Press, 1986.
[21] Piper DC, Upton N, Smith MI, Hunter J. The novel brain neuropeptide, orexin A, modulates the sleep–wake cycle of rats. Eur J Neurosci 2000;12:726–30.
[22] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Wu-Schyong L, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors. A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. Cell 1998;92:573–85.
[23] Sato-Suzuki I, Kita I, Oguri M, Arita H. Stereotyped yawning responses induced by electrical and chemical stimulation of paraventricular nucleus of the rat. J Neurophysiol 1998;80:2765–75.
[24] Sawchenko PE, Swanson LW. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J Comp Neurol 1982;205:260–72.
[25] Siegel JM. Narcolepsy: a key role for hypocretins (orexins). Cell 1999;98:409–12.
[26] Sweet DC, Levine AS, Billington CJ, Kotz CM. Feeding response to central orexins. Brain Res 1999;821:535–8.
[27] Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LHT, Guan X-M. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 1998;438:71–5.