Stereotyped Yawning Responses Induced by Electrical and Chemical Stimulation of Paraventricular Nucleus of the Rat

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Sato-Suzuki, Ikuko, Ichiro Kita, Mitsugu Oguri, and Hideho Arita. Stereotyped yawning responses induced by electrical and chemical stimulation of paraventricular nucleus of the rat. *J. Neurophysiol.* 80: 2765–2775, 1998. Yawning was evoked by electrical or chemical stimulation in the paraventricular nucleus (PVN) of anesthetized, spontaneously breathing rats. To evaluate physiological aspects of yawning, we monitored polygraphic measures as follows; a coordinated motor pattern of yawning was assessed by monitoring breathing [intercostal electromyogram (EMG)], mouth opening (digastric EMG), and stretching of the trunk (back EMG). We also recorded blood pressure (BP), heart rate, and the electrocorticogram (ECoG) to evaluate autonomic function and arousal responses during yawning. A stereotyped yawning response was reproducibly evoked by electrical stimulation or microinjection of L-glutamate or NOC-7, a nitric oxide (NO)-releasing compound, into the PVN. The stereotyped yawning response consisted of two sequential events, an initial response represented a depressor response and an arousal shift in the ECoG to lower voltage and faster rhythms. These initial changes were followed by a yawning behavior characterized by a single large inspiration with mouth opening and stretching of the trunk. A similar sequence of events occurred during spontaneous yawning; a fall in BP and ECoG arousal preceded a yawning behavior. An increase in the frequency of spontaneous yawns was also observed after microinjection of L-glutamate or NOC-7 into the PVN. Intravenous administration of N^G-monomethyl-L-arginine, an inhibitor of nitric oxide synthase (NOS), prevented the stereotyped yawning response evoked by chemical stimulation of the PVN. Histological examination revealed that effective sites for the yawning responses were located in the medial part of the rostral PVN, the site of parvocellular and magnocellular neurons. NADPH-diaphorase histochemistry showed the existence of NOS-containing cells in yawning-evoked sites of the PVN. In summary, the sequential events of yawning may be generated by NOS-containing parvocellular neurons in the medial part of the rostral PVN projecting to the lower brain stem.

INTRODUCTION

The paraventricular nucleus (PVN) of the hypothalamus is essential for the occurrence of yawning as demonstrated by Argiolas et al. (1987). They found that microinjection of several substances, including apomorphine, into the PVN increases the frequency of spontaneous yawns (Melis et al. 1986, 1987) and electrical lesion of the PVN prevents yawning responses induced by apomorphine (Argiolas et al. 1987). These data were obtained mainly by counting the number of mouth openings in conscious rats.

However, 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 (Bertolini and Gessa 1981; Lehmann 1979; Urbá-Holmgren et al. 1977). Yawning is also accompanied by changes in autonomic function, such as lacrimation and erection (Heusner 1946; Melis et al. 1986, 1987). Furthermore, yawning is a phenomenon that subserves arousal (Concu et al. 1974). It is therefore of interest to examine whether these various events of yawning could be evoked simultaneously by stimulation in the PVN, on which there is little information. To evaluate various physiological aspects of yawning, we monitored polygraphic measures representing a yawning response in anesthetized, spontaneously breathing rats. A coordinated motor pattern of yawning was assessed by monitoring breathing [intercostal electromyogram (EMG)], mouth opening (digastric EMG), and stretching of the trunk (back EMG). Autonomic function was evaluated by measuring blood pressure (BP) and heart rate (HR). The autonomic measures were necessary because the PVN plays a significant role in cardiovascular regulation (Kannan et al. 1988; Wardrop and Porter 1995). We also recorded the electrocorticogram (ECoG) to determine the arousal response during yawning. We demonstrated that a stereotyped yawning response was reliably evoked by each stimulation of the PVN. We focused this study on features of the stereotyped yawning response.

To pinpoint responsive sites in the PVN, we used a combination of electrical stimulation and microinjection of L-glutamate procedures. By using a method of electrical stimulation, we systemically searched for sites in and around the PVN from which a yawning response was evoked. Then we microinjected L-glutamate in responsive sites to verify that responses were caused by neuronal cell bodies rather than fibers of passage (Goodchild et al. 1982).

We further studied the potential contribution of nitric oxide (NO) to stereotyped yawning responses. In this connection, Melis and Argiolas (1993, 1995) previously reported that NO in the PVN is an important factor influencing the frequency of spontaneous yawns. We performed nicotinamide adenine dinucleotide phosphate diaphorase (NADPH) staining to evaluate whether nitric oxide synthase (NOS)-positive neurons are localized in responsive sites for yawning. We then microinjected NOC-7, an NO-releasing compound, into the PVN and also examined whether yawning

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responses were suppressed by a NOS inhibitor [N^G-monomethyl-L-arginine (L-NMMA)].

METHODS

Experiments were performed on 22 male Wistar rats weighing 350–450 g. The rats were anesthetized with 30 mg/kg pentobarbital sodium intraperitoneally, and additional doses were given as needed. Catheters were placed in the femoral artery to monitor arterial BP and in the femoral vein for injections. HR was measured from the BP pulse with a tachometer (AT-601G, Nihon Kohden). To monitor respiratory activity, a pair of twisted wire electrodes, insulated except for 1 mm at the tips, was implanted into the lower intercostal space, by way of a 23-gauge hypodermic 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 and into the back muscle to monitor stretching activity. For ECoG recordings, holes were drilled in the skull, and two screw electrodes were implanted. These polygraphic signals were all stored in a DAT data recorder (PC208Ax, Sony) for further analysis. ECoG waves were processed by power spectra analysis to determine the arousal level by using the Mac Lab System (AD Instruments). Rectal temperature was maintained at 37°C with a heating lamp.

The animal was fixed prone in a stereotaxic frame. A parietal craniotomy was performed, and the dura was opened to advance electrodes. For electrical stimulation and drug microinjection, we used a bipolar concentric microelectrode with a central opening (0.5 mm OD, 0.2 mm ID) connected to a fine cannula. The free end of the cannula was attached to a 1.0 μl microsyringe or a picopump (PV830 Pneumatic, WPI) for injections. The cannula was filled with either 0.1 M L-glutamate (Wako, Japan) or 0.1 M NOC-7 (Dojindo, Japan). L-Glutamate was dissolved in artificial cerebrospinal fluid (147 mN NaCl, 3 mM $NaHCO_3$, 3.5 mM KCl, 1.3 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.51 mM NaH_2PO_4 and 1.1 mM urea, pH 7.30–7.35). NOC-7 was selected as an NO-releasing compound in this study because of its feature of generating NO spontaneously without any coproducts and its clearly defined half life ($t_{1/2}$ = 1.7 min). NOC-7 crystal was freshly dissolved in 0.01 N NaOH solution. As a control for L-glutamate, the same amount of artificial cerebrospinal fluid was injected, and, as a control for NOC-7, the same amount of 0.01 N NaOH solution was injected in responsive sites (3 rats). A small air bubble in a fine cannula (0.5 mm OD) allowed calculation of the microinjection volume by measuring the movement of the air bubble. L-NMMA (Sigma), a potent inhibitor of NOS, was injected via the femoral vein.

Electrical stimulation consisted of constant-current train pulses, 20–100 μA in intensity and 0.5 ms in duration, delivered for 10 s at 50 Hz. Electrical stimulation trains were systemically delivered to an area 0.2–0.3 mm lateral to the midline and 1.1–1.4 mm posterior to the bregma and 5.7–7.0 mm vertical from the dura, according to the Paxinos and Watson atlas of the rat brain (1986). After repeated exploratory tracking, we could identify sites where maximal yawning responses were obtained. A small volume (0.1–0.2 μl) of either L-glutamate or NOC-7 was then injected into the same responsive site to evaluate whether a cell's excitability could be increased. On completion of the experiment, successful recording sites were marked by making electrolytic lesions (1 mA for 20 s) for histological examination. The rat was then deeply anesthetized with pentobarbital sodium (50 mg/kg) to remove the brain.

For NADPH-diaphorase histochemistry, the rats were deeply anesthetized with pentobarbital sodium (50 mg/kg) and perfused through the left cardiac ventricle with 300 ml of Tyrode solution, followed by Zamboni's fixative (300 ml) containing 4% paraformaldehyde and 0.2% picric acid in 0.15 M phosphate buffer (PB, pH 7.4). The brains were then removed, postfixed in the same fixative for 2 days, and immersed in solutions containing graded concentrations (5, 10, and 30%) of sucrose in 0.1 M PB at 4°C. Transverse 40-μm-thick sections were made serially on a freezing microtome and collected in 0.1 M PB. The sections were then immersed in 0.1 M PB containing 1 mg/ml nitrobluetetrazolium, 0.3 mg/ml β-NADPH, and 0.3% Triton X-100 and incubated at 37°C for 1 h. They were then rinsed in distilled water and air dried.

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 a level for significance. Values are expressed as the mean ± SD.

RESULTS

Stereotyped yawning responses induced by electrical and chemical stimulation of the PVN

After repeated exploratory tracking in the PVN by electrical stimulation, we identified responsive sites where a stereotyped yawning response was reliably elicited. The response consisted of two sequential events, namely initial changes in BP, EMGJAW, EMGBACK, and ECoG recordings, followed by a final yawning event, i.e., a single large inspiration with mouth opening. A detailed description of the response pattern will be shown. A unique feature characterized the stereotyped yawning response. The final yawning event (a single large inspiration) always appeared with a latency of 11.1 ± 2.8 s (n = 15) after onset of electrical stimulation. To rule out the possibility that the long latency response might result from the distance between the electrode tip and responsive neurons, we moved an electrode 0.1-mm steps along the effective track. Furthermore, we systematically searched for responsive sites along other neighboring tracks 0.5 mm rostral, caudal, medial, or lateral to the track from which a stereotyped yawning response was consistently obtained. We confirmed that no other sites could elicit a single large inspiration with shorter delays. A single large inspiration consistently appeared, with a latency of ~11 s.

We then microinjected L-glutamate into responsive sites to confirm that the responses were caused by excitation of cell bodies rather than fibers of passage. We found that each chemical stimulation reproducibly induced a stereotyped yawning response that was qualitatively similar to that evoked by electrical stimulation (Fig. 1); the response pattern was also similar to the sequence of events occurring during a spontaneous yawn. A fall in BP preceded a yawning behavior, i.e., a single large inspiration with mouth opening.

Sequential events of stereotyped yawning evoked by chemical stimulation (Fig. 1, *right panel*) were characterized as follows. During the early phase of yawning, a depressor response and intermittent short bursts of EMGJAW and EMGBACK resulted. In contrast, EMGIC showed little change in amplitude before the occurrence of a single large inspiratory activity. However, a small change in rhythmic activity of EMGIC occurred during the early phase of yawning; there was a small reduction in respiratory rate.

This early phase of the yawning response was followed by a sudden development of a single large inspiration, as evidenced by enhanced inspiratory activity on the EMGIC recording. The single large inspiratory effort was accompa-

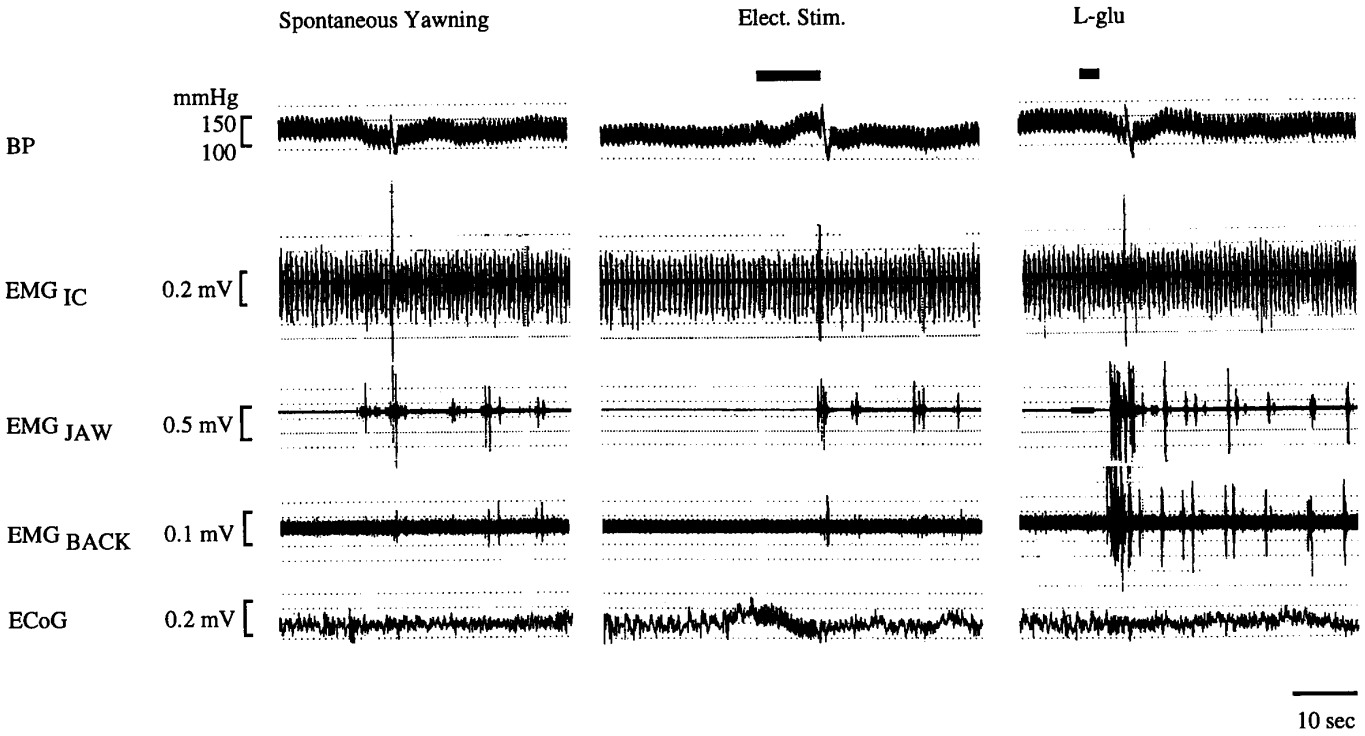

FIG. 1. Representative responses to electrical (*middle panel*) and chemical (*right panel*) stimulation of the paraventricular nucleus (PVN) in an anesthetized, spontaneously breathing rat. Electrical stimulation consisted of constant current (80 μA) train pulses at the rate of 50 Hz for 10 s. Chemical stimulation was performed by microinjection of L-glutamate (0.1 M, 0.2 μl) into the PVN. BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram; EMGBACK, trunk electromyogram; ECoG, cortical electroencephalograph. Analogous changes in the motor pattern, BP and ECoG were observed during spontaneous yawning (*left panel*).

nied by enhanced activities of EMGJAW and EMGBACK, indicating mouth opening and stretching of the trunk. Typical yawning behavior, characterized by a deep inspiration with mouth opening and stretching of the trunk, could be observed visually, although the motion was weak under anesthesia. Concomitantly with the single large inspiration, a notch on the BP recording was observed during the depressor response. After the yawning behavior, intermittent bursts of EMGJAW and EMGBACK occurred. BP returned gradually to control levels.

A similar sequence of events in yawning was observed during a spontaneous yawn (Fig. 1, *left panel*). We could always observe a fall in BP before the final event in yawning, i.e., a yawning behavior characterized by a single large inspiratory activity of EMGIC accompanied by short bursts of EMGJAW and EMGBACK. Several features common to spontaneous yawning and evoked yawning emerged. The patterns of decrease in BP were stereotyped in time course and extent. There were intermittent excitations of EMGJAW and EMGBACK before the occurrence of a final yawning behavior, although lesser in degree during a spontaneous yawn than those observed after chemical stimulation. Yawning behavior was comparable both in recordings of EMGIC, EMGJAW, and EMGBACK and in direct observation.

Power spectral analysis of ECoG waves revealed that an early change in ECoG occurred before a yawning behavior, as shown in Fig. 2. During the control period before chemical stimulation, the ECoG of anesthetized rats was characterized by high voltage and slow waves ($<$1 Hz). Concurrent with the depressor response, ECoG waves shifted to low voltage and fast rhythms (8–9 Hz). After a final yawning behavior, the waves reversed to the control level (slower rhythms).

A similar change in ECoG waves was also found during spontaneous yawning (Fig. 3). As mentioned previously, a fall in BP occurred before a yawning behavior. During this early phase of yawning, ECoG waves shifted to low voltage and fast rhythms (8–9 Hz). After the final yawning behavior, the ECoG waves returned to higher voltage and slower rhythms ($<$1 Hz).

The frequency of spontaneous yawns increased after microinjection of L-glutamate into the PVN (Fig. 4). Spontaneous yawns during the control period before chemical stimulation were very rare, the interval between spontaneous yawns being $\sim$20–30 min (Fig. 4, *top panel*, with control). The second panel in Fig. 4 shows stereotyped yawning responses evoked by electrical stimulation and microinjection of L-glutamate. Thereafter, we noticed that the interval between spontaneous yawns became shorter ($\sim$140 s) at 4 min after microinjection of L-glutamate (Fig. 4, *third panel*). The shortest interval of 70–100 s was observed at 18 min after chemical stimulation in this case (Fig. 4, *bottom panel*). The increase of occurrence was gone in $\sim$40 min after chemical stimulation.

We examined frequency changes of spontaneous yawning in four rats. The minimum interval between spontaneous yawns was 74.3 $\pm$ 9.1 s (n = 4), which was significantly

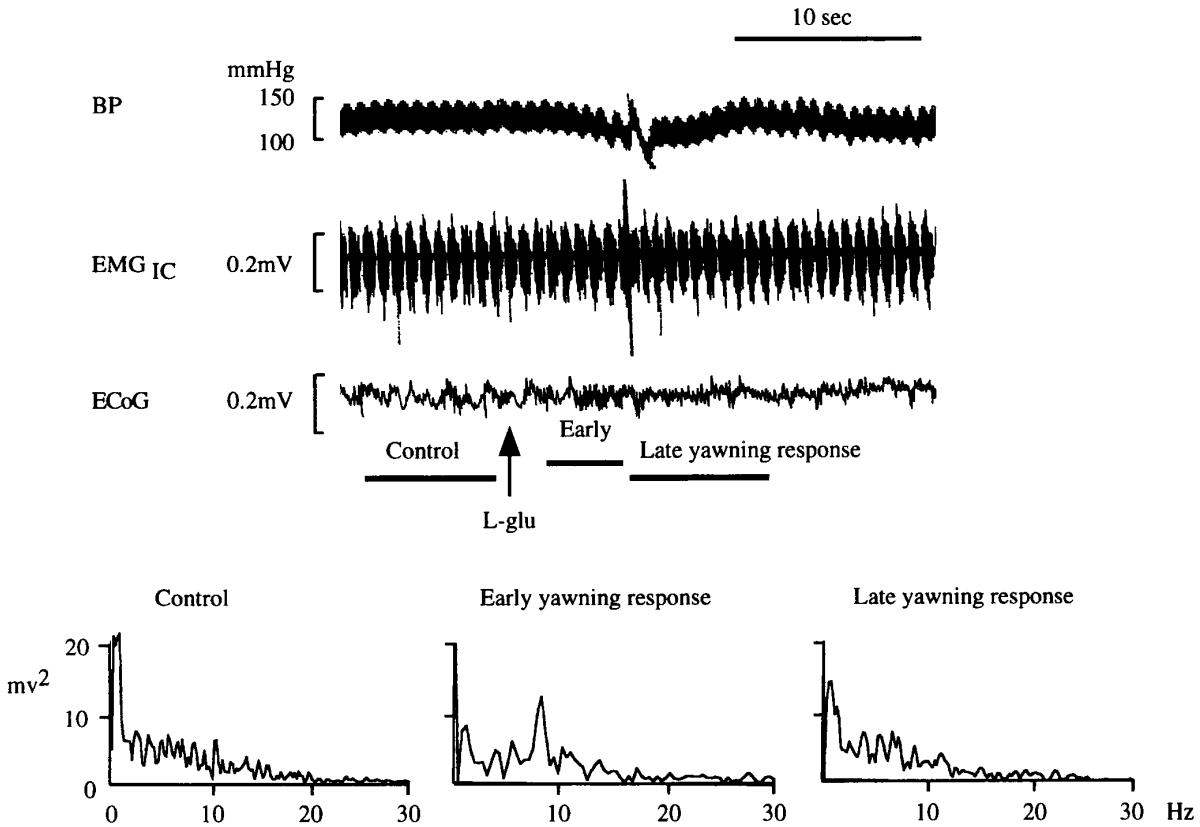

FIG. 2. Typical changes in the electrocorticogram recording during yawning evoked by microinjection of L-glutamate (0.1 M, 0.2 μl) into the PVN. BP, blood pressure; EMGIC, intercostal electromyogram; ECoG, cortical electroencephalograph. An anesthetized rat showed higher voltage and slower waves (<1 Hz). The waves shifted to lower voltage and faster rhythms (8–9 Hz) immediate before yawning. After yawning, the waves completely reversed to slower rhythms.

shorter ($P < 0.05$) than the interval of 1,102 ± 198 s ($n = 4$) obtained during the control period.

Higher threshold for a single large inspiration

To determine thresholds for various events in yawning responses, we raised the current intensities of stimulation in a graded fashion. A typical example is shown in Fig. 5. At an intensity of 30 μA, there were no changes in any of the measures. At an intensity of 50 μA, a depressor response and intermittent bursts of EMGJAW appeared, although no change was observed in EMGIC rhythmic activity. A single large inspiratory activity occurred ~30 s after electrical stimulation, but it was unclear whether this yawning behavior represented a spontaneous event or a long latency response after electrical stimulation. At an intensity of 70 μA, a typical stereotyped yawning response was obtained, namely, an initial change in BP (a depressor response) and intermittent EMGJAW bursts, followed by a final yawning behavior, i.e., a single large inspiration with mouth opening. The threshold for the single large inspiration was higher than that for the depressor response or EMGJAW activation. When the intensity of electrical stimulation was further elevated to 100 μA, a pressor, instead of a depressor, response was elicited, although EMGIC and EMGJAW showed the same responses as those obtained at 70 μA electrical stimulation. The shift from a depressor to a pressor response was consistently obtained in this study when current intensities of stimulation increased in a graded fashion.

The threshold for a single large inspiration was 59.6 ± 16.3 μA ($n = 12$), which was significantly higher ($P < 0.05$) than those for depressor responses, 35.8 ± 14.3 μA ($n = 12$), and activation of EMGJAW, 38.6 ± 15.8 μA ($n = 11$).

Distribution of responsive sites

Light microscopic examination revealed that responsive sites were located in the medial part of the rostral PVN (Fig. 6). No responsive sites for yawning were identified in the lateral part of the rostral PVN or in the caudal PVN. Histological examination demonstrated that both parvocellular and magnocellular neurons intermingled in the responsive sites for yawning.

NADPH-diaphorase staining

We determined whether neurons in responsive sites of the medial part of the rostral PVN were NOS positive by a method of NADPH-diaphorase staining. NOS-positive cells were densely determined within the PVN. We found that the stained area was located just ventral to the region where we made electrolytic lesion as a responsive site for yawning (Fig. 7).

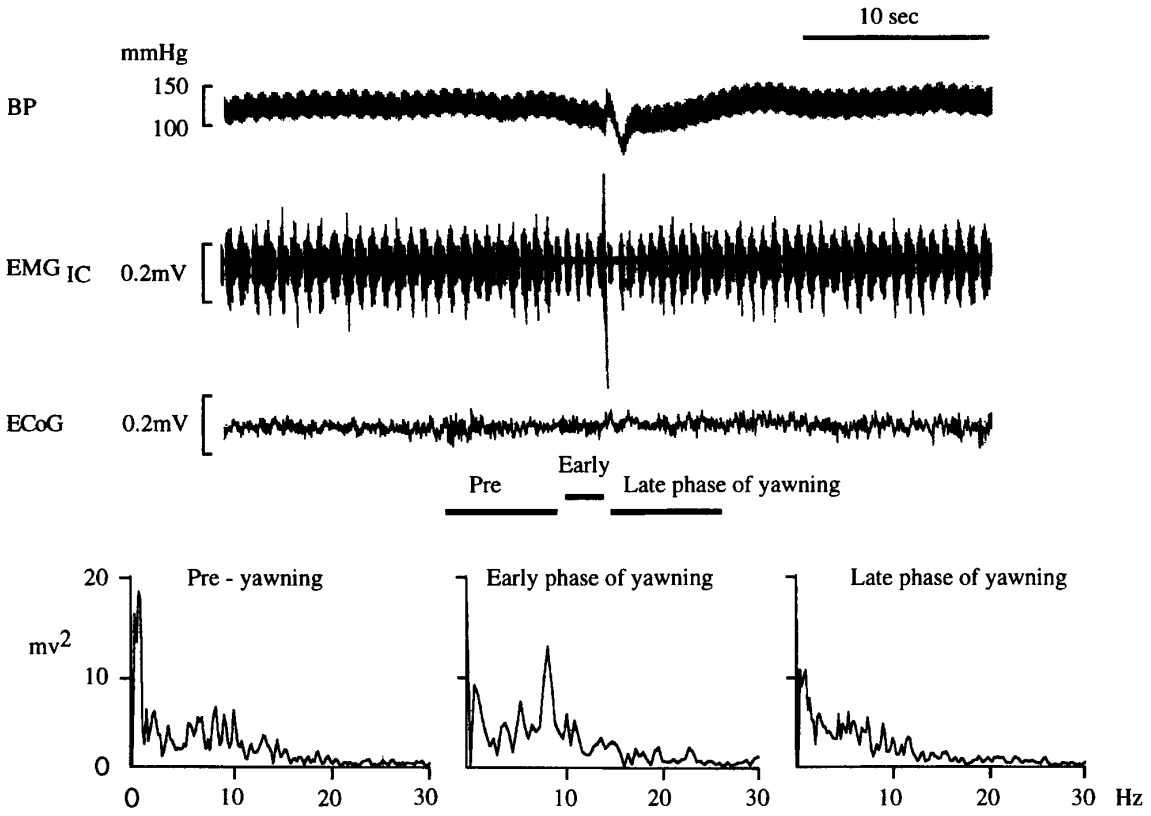

FIG. 3. Typical changes in the ECoG recording during spontaneous yawning. BP, blood pressure; EMGIC, intercostal electromyogram; ECoG, cortical electroencephalograph. An anesthetized rat showed higher-voltage and slower waves (<1 Hz). The waves shifted to lower voltage and faster rhythms (8–9 Hz) immediately before yawning. After yawning, the waves completely reversed to higher voltage and slower rhythms.

Effects of NOC-7 on yawning

We further examined effects of an NO-releasing compound (NOC-7) on yawning. Microinjection of NOC-7 into the PVN elicited a stereotyped yawning response (Fig. 8). The response was analogous to that evoked by microinjection of L-glutamate into the PVN except for the BP response. NOC-7 produced a biphasic change in BP, namely, an initial transient increase in BP followed by a depressor response, whereas L-glutamate induced a depressor response alone. There was a prolonged increase in BP after a yawning behavior when NOC-7 was microinjected into the PVN. We found no significant difference in the activities of EMGIC, EMGJAW, or EMGBACK between NOC-7 and L-glutamate.

The frequency of spontaneous yawns increased by microinjection of NOC-7 in the PVN, a phenomenon similar to that evoked by L-glutamate. A typical example is shown in Fig. 9. After microinjection of NOC-7 into the PVN, the interval between spontaneous yawns gradually became shorter. The shortest interval of 50–70 s was observed at 12 min after NOC-7 microinjection (Fig. 9, *bottom panel*), whereas the interval between spontaneous yawns during the control period was ~300 s (Fig. 9, *top panel*). The increased frequency of spontaneous yawns returned to the control level in ~25 min after NOC-7 microinjection.

Effects of L-NMMA on the yawning response

We finally examined the effects of a NOS inhibitor (L-NMMA) on yawning responses evoked by microinjection of L-glutatmate into the PVN. Microinjection of L-glutatmate into the PVN before intravenous injection of L-NMMA induced a yawning response (Fig. 10, *left panel*). This response was inhibited by intravenous administration of L-NMMA (1.5 mg/kg; Fig. 10, *middle panel*). At 3 min after L-NMMA, microinjection of L-glutamate failed to evoke a yawning response. At 10 min after L-NMMA (Fig. 10, *right panel*), a yawning response was evoked by microinjection of L-glutamate into the PVN. Statistical analysis revealed that an inhibitory effect of L-NMMA on yawning responses lasted for 14.5 ± 3.9 min ($n = 6$).

DISCUSSION

The main finding was that a stereotyped yawning response was reproducibly evoked by microinjection of L-glutamate or NOC-7 into the PVN. The stereotyped yawning response consisted of sequential events; a single large inspiration with mouth opening occurred after a depressor response and ECoG arousal shift. Earlier experimental studies on yawning by other investigators (Melis et al. 1986, 1987; Van Erp et al. 1990) showed that chemical stimulation of the PVN increases the frequency of spontaneous yawns in conscious rats. A similar increase in the frequency of spontaneous yawns after chemical stimulation was also observed, although the data were obtained in anesthetized, spontaneously breathing rats. However, a new finding from this study was the appearance of stereotyped yawning responses. We there-

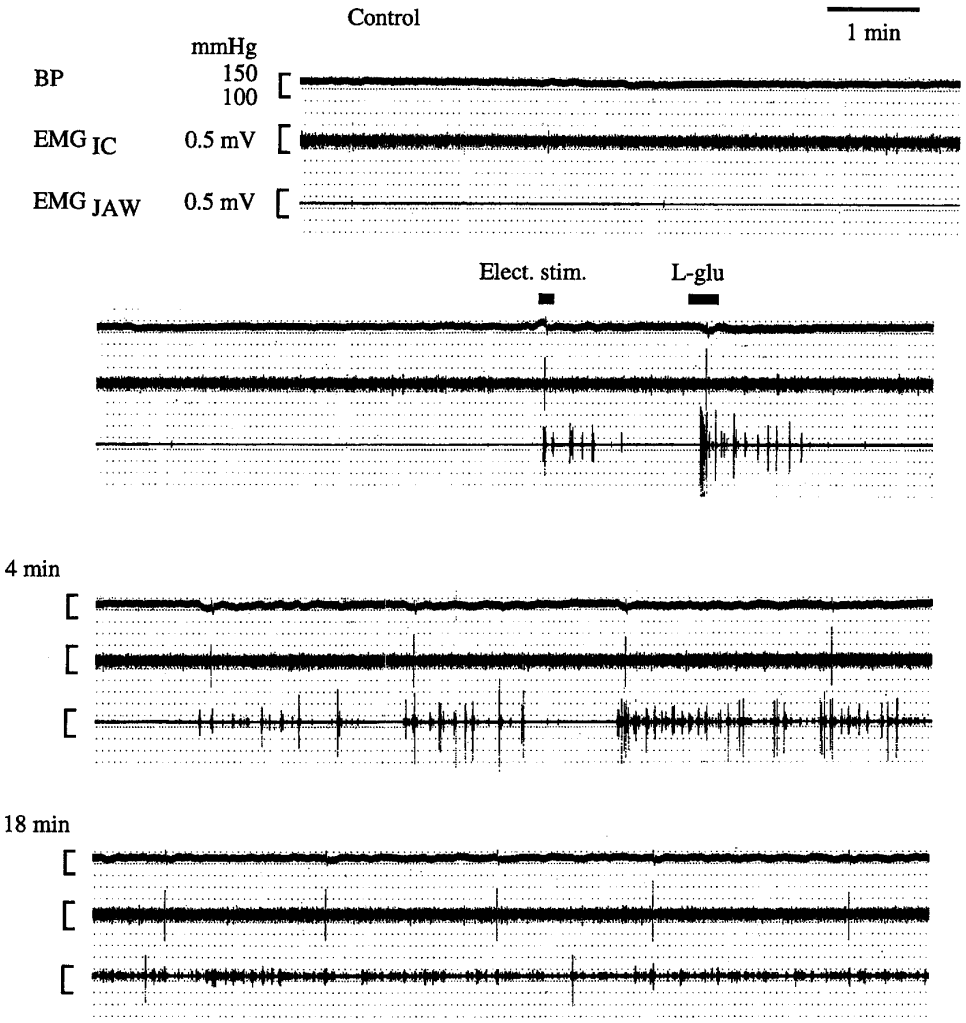

FIG. 4. Typical example showing the effect of microinjection of L-glutamate into the PVN on yawning. Microinjection of L-glutamate (0.1 M, 0.2 μl) increased the frequency of spontaneous yawn. BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram.

fore focus the following discussion on features of these stereotyped responses.

This study demonstrated a unique aspect of the stereotyped yawning response; a single large inspiration consistently occurred with a long latency (~11 s) after electrical stimulation. The delay in the response is not attributable to the distance between the electrode tip and the responsive cells because we could not obtain a single large inspiratory effort with a shorter delay at any region near the site where yawning was reliably evoked. It is also unlikely that the delayed response is due to the time required for axonal–synaptic transmission from the PVN to the lower brain stem where various types of respiratory neurons are located (Cohen 1979) because we could observe a small, but consistent, change in rhythmic activity of the EMGIC before the occurrence of a large inspiratory effort. We therefore hypothesize that the long latency results from the time required for neuronal processes to generate a unique motor pattern of a single large inspiration. An analogous example can be found in respiratory-related motor patterns occurring in sneezing (Nonaka et al. 1990) or vomiting (Miller et al. 1987). Such respiratory-related motor outputs appear with a considerable latency after stimulation is delivered to the afferent pathways.

A most interesting aspect of these findings is that yawning consists of sequential events; a single large inspiration with mouth opening (yawning behavior) always occurred after the depressor response and the ECoG arousal shift. This timing sequence was consistent during all types of yawning, namely, spontaneous yawning, and yawning responses induced by chemical (L-glutamate and NOC-7) or electrical stimulation of the PVN. This finding indicates that a neuronal structure exists in the PVN, which triggers the sequential events of yawning. On the other hand, the finding that this stereotyped yawning response represents various behavioral and autonomic events suggests that, at this level of the PVN, the origins of various outputs are integrated. Taken together, we hypothesize that a neuronal structure triggering yawning might be linked to the origins of at least five distinct efferents projecting to the respiratory, cardiovascular, facial motor, spinal motor, and arousal systems. Nevertheless, it remains undetermined whether the neuronal structure triggering the stereotyped yawning response consists of a single or different cell type.

The stereotyped yawning response observed here might be mediated by the oxytocinergic parvocellular neurons projecting to the lower brain stem, based on the following data. First, Melis et al. (1996) demonstrated that oxytocinergic

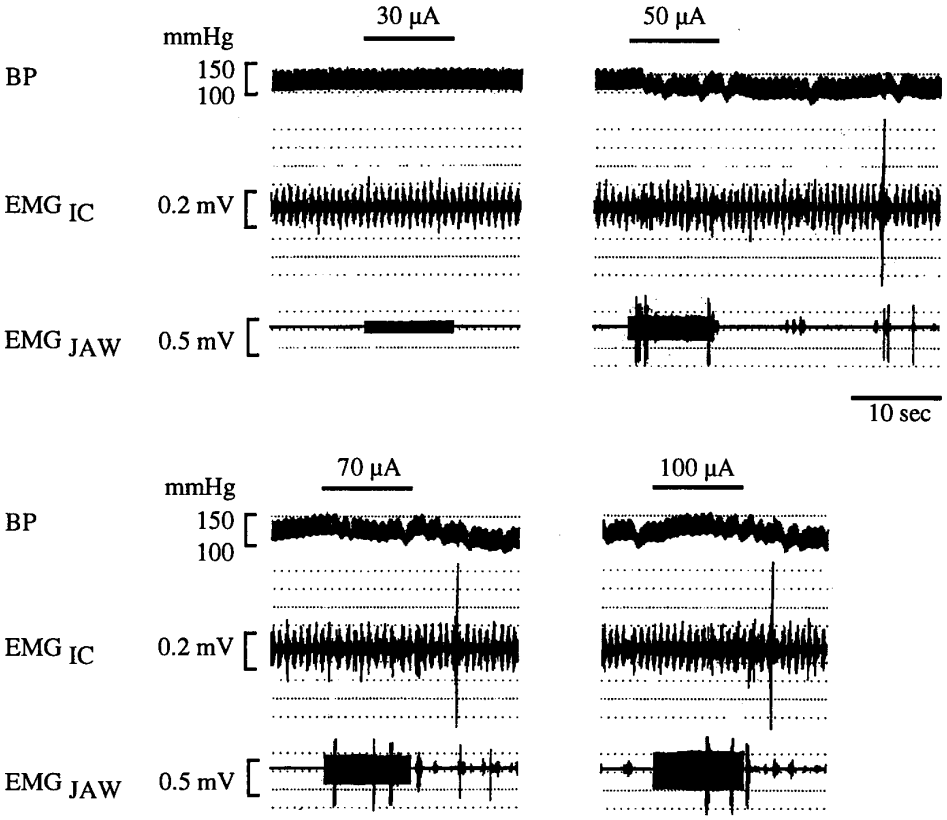

FIG. 5. Typical example showing the difference in the threshold for each measure. Electrical stimulation consisted of constant current (30, 50, 70, and 100 μA) train pulses at the rate of 50 Hz for 10 s. The responses to BP differed with the current intensity of the electrical stimulation. BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram.

cells within the PVN are responsible for yawning. Second, Sawchenko and Swanson (1982) reported that oxytocinergic parvocellular cells in the PVN send descending axons to the lower brain stem, including the locus coeruleus, nucleus of the solitary tract, the dorsal motor nucleus of the vagus nerve, ventrolateral medulla, and the spinal cord (Swanson 1987). These regions are involved in arousal, respiratory, cardiovascular, and other autonomic functions. Third, our histological examination showed that the effective sites of the stereotyped yawning response were located at the boundary zone of parvocellular and magnocellular neurons in the medial part of the rostral PVN. It is unlikely that the magnocellular neurons are involved in yawning because magnocellular neurons with oxytocin are generally known to play a major role in neuroendocrinal function (Torres et al. 1993) rather than behavioral control. Taken together, we speculate that the stereotyped yawning response may be mediated by oxytocinergic parvocellular neurons in the medial part of the rostral PVN projecting to the lower brain stem and the spinal cord.

To our knowledge, this is the first demonstration that a depressor response always precedes yawning behavior, i.e.,

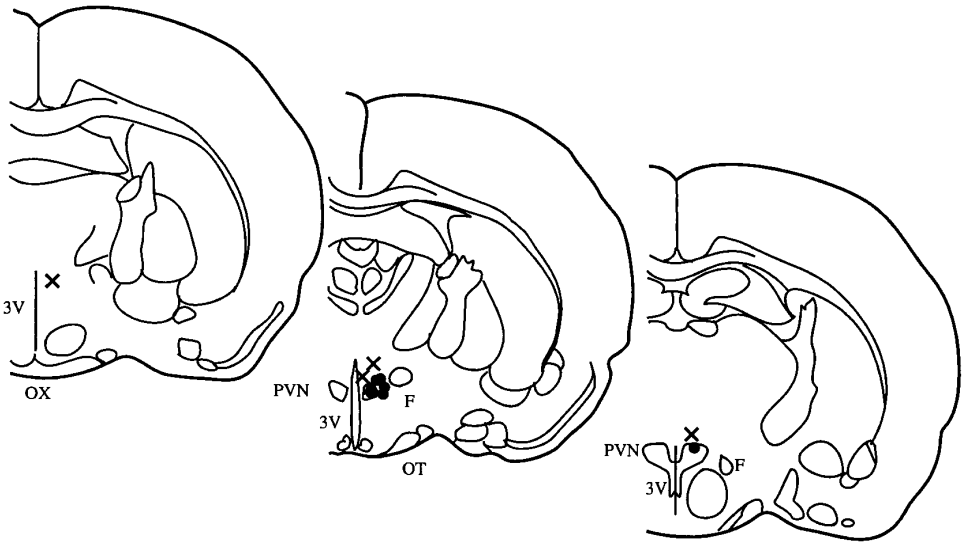

FIG. 6. Schematic drawings of transverse sections of the brain, illustrating locations of 10 sites effective for yawning. Filled circles: responsive sites. Crossed symbols: nonresponsive sites.

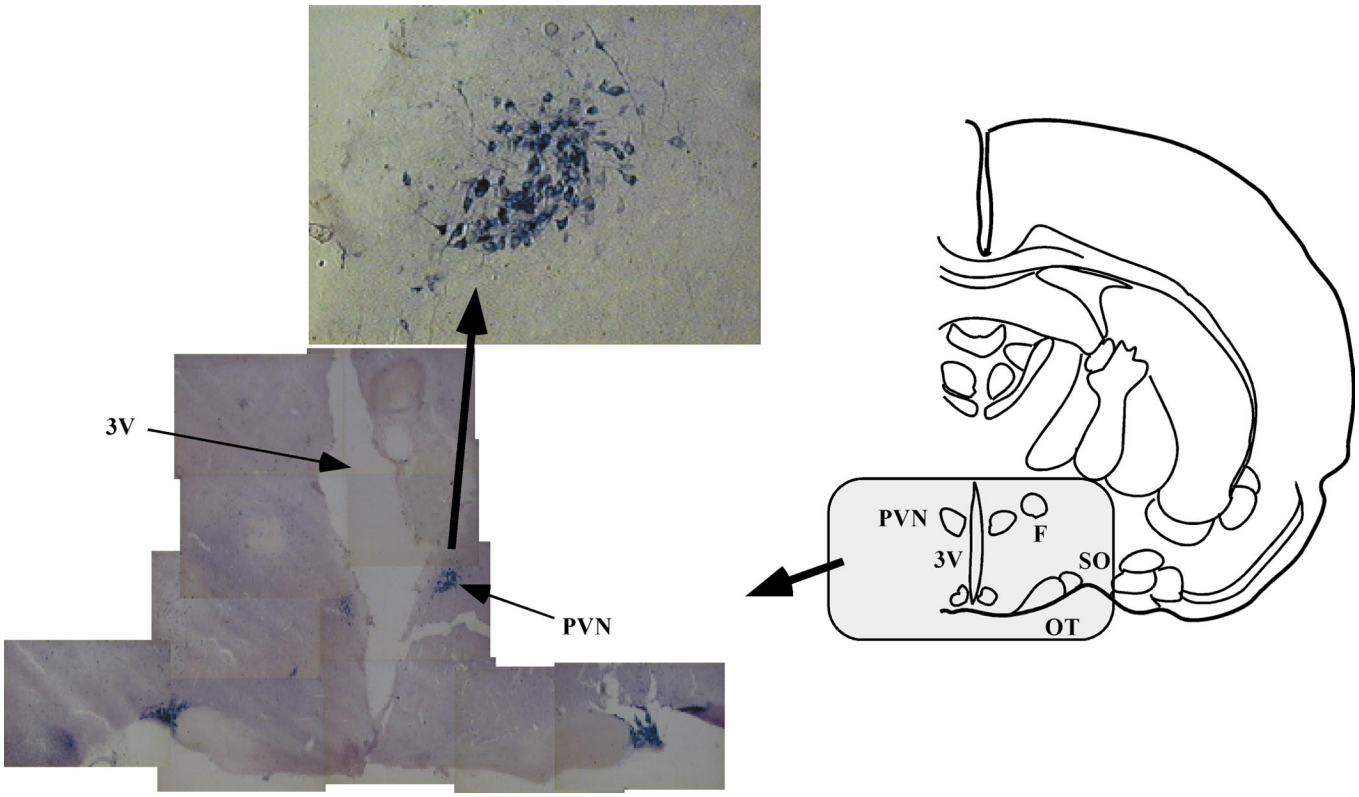

FIG. 7. Histological examination of NADPH-diaphorase positive cells in the PVN. The responsive sites were located in the medial part of the rostral PVN, which was consistent with those for the electrolytic lesions.

a single large inspiration with mouth opening. These sequential events in yawning occurred during either spontaneous yawning or yawning responses induced by L-glutamate or NOC-7. This phenomenon is of particular interest for examining the relationship between the occurrence of yawning and attenuation of cerebral circulation. A relevant example is the case of orthostatic hypotension (Shvartz 1996). A sudden postural change from the supine to upright position results in fainting in some subjects and is explained as a failure to regulate cerebral circulation. Although some subjects may not faint, others occasionally show frequent yawning and dizziness, indicating that yawning is one symptom developed by attenuation of cerebral circulation. An interesting case report about a patient with repetitive yawning (Askenasy and Askenasy 1996) shows that falls in BP and inhibition of sympathetic nerve activity occurred with yawn-

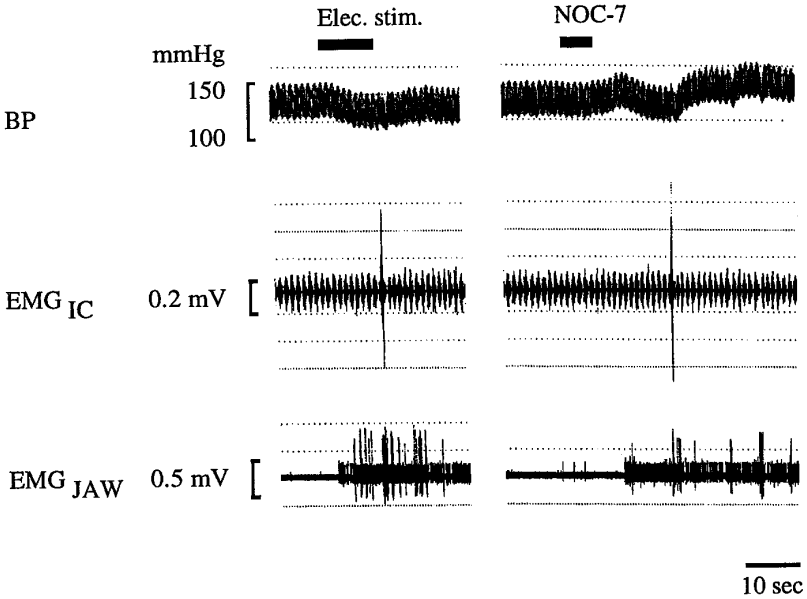

FIG. 8. Representative responses to microinjection of NOC-7 into the PVN in an anesthetized, spontaneously breathing rat (*right panel*). Microinjection of NOC-7 (0.1 M, 0.2 μl) evoked a yawning response that was qualitatively the same as that evoked by electrical stimulation (80 μA, *left panel*). BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram.

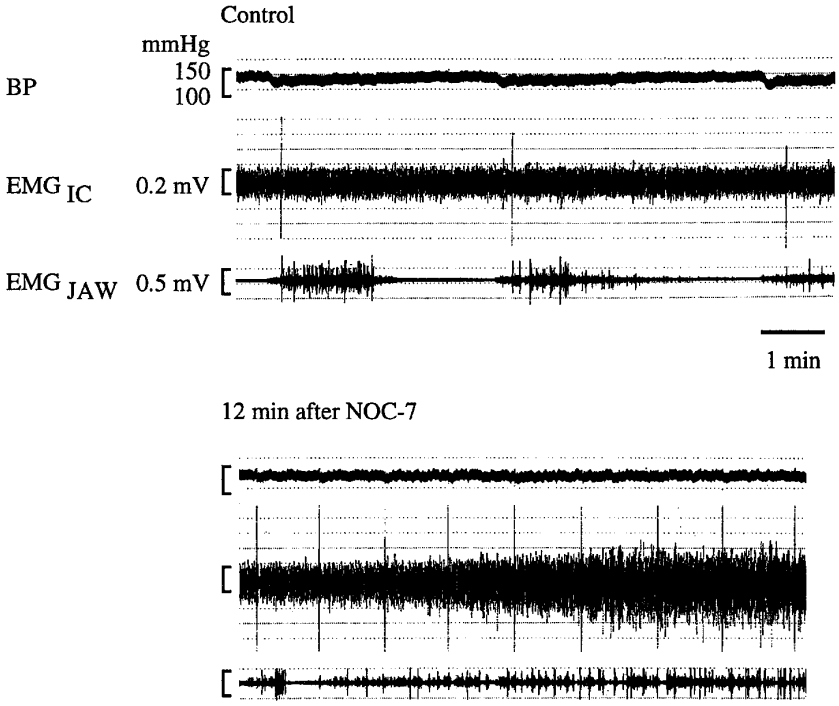

FIG. 9. Effects of microinjection of NOC-7 into the PVN on the frequency of spontaneous yawns. Microinjection of NOC-7 (0.1 M, 0.2 μl) increased the frequency of spontaneous yawns 12 min after injection. BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram.

ing. This clinical observation is consistent with our finding that a decrease in BP always preceded the final yawning behavior.

Our finding that the depressor response derives from stimulation of the medial part of the rostral PVN is supported by previous investigations reported by Kannan et al. (1988) and Porter and Brody (1986). They demonstrated that two functionally different sites exist for cardiovascular control in the PVN. Depressor (sympathoinhibitory) responses are elicited by chemical and electrical stimulation in the rostromedial part of the PVN, whereas pressor (sympathoexcitatory) responses are evoked by stimulation of the caudal PVN. Our results support these data. Our data further demonstrated that activation of the medial part of the rostral PVN evoked not only depressor responses but also yawning behavior.

This study further demonstrated that PVN neurons mediating stereotyped yawning responses were NOS positive, as revealed by NADPH-diaphorase histochemistry. This histochemical observation is in good agreement with recent de-

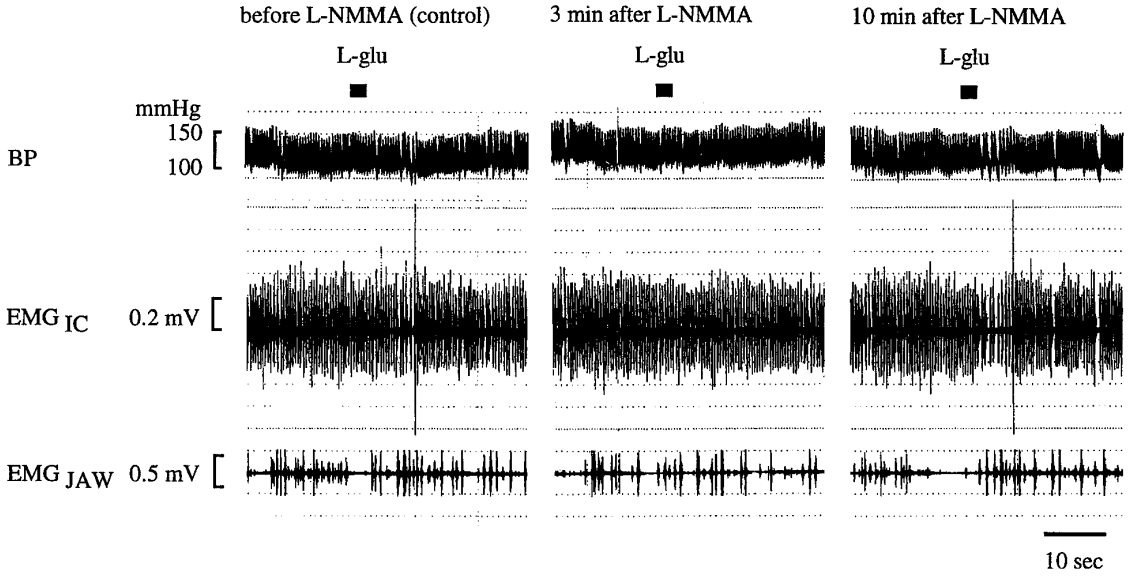

FIG. 10. Effects of N^G-monomethyl-L-arginine (L-NMMA) on yawning evoked by chemical stimulation of the PVN. Intravenous administration of L-NMMA (1.5 mg/kg) prevented the yawning response. Chemical stimulation consisted of microinjection of L-glutamate (0.1 M, 0.2 μl) into the PVN. BP, blood pressure; EMGIC, intercostal electromyogram; EMGJAW, digastric electromyogram.

tailed studies showing that the PVN is one of the brain areas containing the highest number of NOS neurons (Bredt et al. 1990; Southam and Garthwaite 1993). Within the PVN, NOS exists in the magnocellular cells (Hatakeyama et al. 1997) as well as parvocellular cells (Kishimoto et al. 1996; Yamada et al. 1996). As mentioned previously, we propose the concept that the stereotyped yawning response might be mediated by oxytocinergic, parvocellular neurons projecting to the lower brain stem. Taken together, these findings suggest that NOS might coexist in those neurons.

In the physiological experiments, yawning responses were also induced by microinjection of NOC-7, an NO-releasing compound, into the PVN. The features of the stereotyped yawning responses were essentially the same as those evoked by L-glutamate. This finding further nominated NO as an effective neuronal chemical that generates yawning. Moreover, the inhibition of NOS by intravenous administration of L-NMMA prevented the yawning response evoked by microinjection of L-glutamate into the PVN, indicating that endogenous NO in the PVN plays a role in generation of yawning. These data suggest that the mechanism underlying the yawning responses evoked by L-glutamate and NO is a common one that is linked. In this connection, Bredt and Snyder (1989) established that activation of the L-glutamate (NMDA) receptor leads to production of a second messenger that is identified as NO in NOS-containing cells. Taking these aspects into consideration, we hypothesize that stimulation of NMDA receptor activates NOS and produces NO; this neuronal process will finally generate yawning. The same hypothesis was recently presented in the review by Melis and Argiolas (1997).

ECoG arousal 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. Concu et al. (1974) also demonstrated that EEG arousal and yawning appeared concomitantly; however, our data differ slightly in that EEG arousal appeared not during the yawning but before the yawning behavior (a single large inspiration with mouth opening). Concu et al. (1974) suggested that EEG arousal does not result from stretching the trunk. We further suggest the possibility that the medial part of the rostral PVN might underlie the arousal.

In summary, microinjection of L-glutamate or an NO-releasing compound into the medial part of the rostral PVN induced a stereotyped yawning response, a single large inspiration with mouth opening that occurred after the depressor response and ECoG arousal shift. This stereotyped pattern was also observed during spontaneous yawning. We hypothesize that the sequential events of yawning may be triggered by parvocellular neurons with NOS in the medial part of the rostral PVN, which send descending axons projecting to respiratory, cardiovascular, motor, and arousal systems in the lower brain stem.

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