Decreased CSF pH at ventral brain stem induces widespread c-Fos immunoreactivity in rat brain neurons

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Douglas, R. M., C. O. Trouth, S. D. James, L. M. Sexcius, P. Ke, O. Dehkordi, E. R. Valladares, and J. C. McKenzie. Decreased CSF pH at ventral brain stem induces widespread c-Fos protein immunoreactivity in rat brain neurons. J Appl Physiol 90: 475–485, 2001.—Physiological evidence has indicated that central respiratory chemosensitivity may be ascribed to neurons located at the ventral medullary surface (VMS); however, in recent years, multiple sites have been proposed. Because c-Fos immunoreactivity is presumed to identify primary cells as well as second- and third-order cells that are activated by a particular stimulus, we hypothesized that activation of VMS cells using a known adequate respiratory stimulus, H+, would induce production of c-Fos in cells that participate in the central pH-sensitive respiratory chemoreflex loop. In this study, stimulation of rostral and caudal VMS respiratory chemosensitive sites in chloralose-urethane-anesthetized rats with acidic (pH 7.2) mock cerebrospinal fluid induced c-Fos protein immunoreactivity in widespread brain sites, such as VMS, ventral pontine surface, retrotrapezoid, medial and lateral parabrachial, lateral reticular nuclei, cranial nerves VII and X nuclei, A1 and C1 areas, area postrema, locus coeruleus, and paragigantocellular nuclei. At the hypothalamus, the c-Fos reaction product was seen in the dorsomedial, lateral hypothalamic, supraoptic, and periventricular nuclei. These results suggest that 1) multiple c-Fos-positive brain stem and hypothalamic structures may represent part of a neuronal network responsive to cerebrospinal fluid pH changes at the VMS, and 2) VMS pH-sensitive neurons project to widespread regions in the brain stem and hypothalamus that include respiratory and cardiovascular control sites.

chemosensitivity; immunocytochemistry

EXPERIMENTAL INVESTIGATIONS have implicated neurons located at the ventrolateral medullary surface (VMS) in the central regulation of both cardiovascular and respiratory activity (2, 19, 20, 35). Some of these neurons are believed to be involved in the central CO2 pH chemoreceptor drive to respiration in mammals (20, 31). Lesioning or blockade of discrete areas at the VMS in cats abolishes the respiratory sensitivity to inspired CO2 (31). Earlier, Berndt et al. (2) reported that electrical stimulation of the caudal chemosensitive area [caudal VMS (cVMS)] in peripheral chemoreceptor-denervated cats caused hyperventilation. However, when procaine was applied topically to the cVMS during electrical stimulation, apnea resulted, and this was only reversed when direct respiratory center stimulation was applied. It was, therefore, postulated that the integrity of the VMS chemosensory neuronal elements was essential to the respiratory center drive in the absence of peripheral chemoreceptor afferents. Recent hypotheses concerning central respiratory chemoreception have been expanded to implicate not only the VMS but also the retrofacial nucleus, portions of the nucleus tractus solitarius (NTS), midline raphe structures, and the retrotrapezoid nucleus (RTN) as potential sites of central respiratory chemosensitivity (3, 6, 24, 25, 26).

The precise location of the central chemosensitive neurons that modulate respiratory activity is still being sought, and various techniques have been utilized to identify the exact sites and characteristics of the specific morphological substrates involved. Ideally, a specific marker expressed by neurons that respond to adequate respiratory stimuli could serve to identify the chemoreceptor element and, perhaps, other neurons synaptically coupled to them. In fact, stimulation of neurons within the central nervous system (CNS) is known to initiate cascading biochemical reactions, which may activate immediate early genes. The c-fos gene, which is a transcription modulator, is a DNA binding protein that initiates biochemical long-term adaptive changes in neurons (23, 29). The c-fos and other immediate early genes are believed to act as third messengers in signal transduction by altering gene expression in response to neuronal excitation. Expression of the c-Fos protein, which is translated from the protooncogene c-fos, is a sign of cell activation and has, therefore, been utilized immunocytochemically as a metabolic marker and tracer of activities in the CNS (29).

The present study utilizes the immunocytochemical expression of c-fos as a marker of increased activity in an attempt to localize neuronal elements within the rat
brain that are activated in response to stimulation of the VMS with cerebrospinal fluid (CSF) pH changes. These neurons may represent part of the neuronal network involved in the central pH chemosensory drive to breathing.

METHODS

Adult Sprague-Dawley rats (n = 21; weight 250–350 g) were anesthetized with chloralose-urethane (40 mg/kg α-chloralose and 200 mg/kg urethane ip), and a tracheal cannula was routinely inserted via tracheotomy. In spontaneously breathing animals, the VMS was exposed from the lower pons to about spinal cord C1–C2 level and ~2–3 mm lateral from the midline. Rectal temperature was maintained at 37 ± 1°C.

Chemical Stimulation

Effect of pH changes. In a series of studies, pledgets soaked in mock CSF (mCSF) pH 7.2 (acidic) and 7.4 (control) were applied unilaterally to either the rostral VMS (rVMS) or the cVMS of rats at sites (Fig. 1) analogous to the classic chemosensitive regions described in the cat (20). The composition of the mCSF was as follows (in mM: 121 NaCl, 1.14 CaCl2, 24 HCO3−, and 5 KCl (pH 7.4); the solution was bubbled with 5% CO2 in air and maintained at 37°C). For mCSF pH 7.2, the pH of mCSF was adjusted with HCl while the solution was bubbled with 5% CO2 in air and maintained at 37°C. cVMS and rVMS sites may be identified in the vicinity of the exit of the rootlets of cranial nerves XII and VI, respectively. Both regions extend lateral (1–2 mm) to the pyramids in a region that we believe contains the parapyramidal cell group (PPR). In the rat, cVMS chemosensitive sites appear to extend from the rostral to the caudal extent of the hypoglossal rootlets (~3–5 mm rostrocaudally) as they exit the brain stem, whereas rVMS chemosensitive sites extend 2–3 mm below the pontomedullary border. The pledgets were replaced every 10 min for 2 h to allow for the translation of the gene product, the c-Fos protein. Excess CSF was blotted from the control and acidic mCSF-soaked pledgets before application to the VMS to avoid spillage to surrounding regions. In addition, cottonoid wicks applied bilaterally, at both the rostral and caudal extent of the exposed medulla, served to absorb endogenously generated CSF that might mix with and, therefore, spread the stimulus to adjacent areas.

In this study, we performed three types of control experiments: 1) anesthesia controls, which were perfused immediately on the animal’s succumbing to the anesthetic; 2) surgical controls, which were perfused immediately after the VMS was exposed; and 3) pH 7.4 controls, wherein pledgets containing mCSF at pH 7.4 were applied to the VMS for a 2-h period utilizing the same protocol as for experimental animals. The extent of c-Fos labeling, both in terms of intensity of staining and number of cells stained, was much greater in the experimental animals than in controls (i.e., anesthesia, surgery, and pH 7.4). The number of animals utilized in each of the experimental protocols was as follows: surgery controls, n = 2; pH 7.4 controls at the cVMS, n = 5; pH 7.4 at the cVMS experiments, n = 10; pH 7.4 controls at the rVMS, n = 5; and pH 7.2 at the rVMS experiments, n = 5. The effect of decreased pH was assessed by the immunocytochemical methods described below.

Perfusion and Fixation

The animals were rapidly perfused transcardially with 0.9% saline in 0.1 M phosphate buffer (pH 7.3) and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The fixed tissue was dissected out and placed in fixative for 24 h, followed by cryoprotection in a glycerol-phosphate buffer for 48 h. Subsequently, 40-μm frozen sections were prepared from the entire brain on an American Optical Cryo-Cut II microtome, placed in PBS, and processed for c-Fos immunocytochemistry, according to the procedure of Uemura et al. (36).

c-Fos Immunocytochemistry

The tissues were washed with 3.0–25.0 ml of PBS three times for 10 min each. They were then incubated with 0.3% hydrogen peroxide for 30 min at room temperature. The tissues were washed again with PBS with 0.1% Triton X-100 (TX-100) and incubated with 3.0–10.0 ml PBS containing 0.3% TX-100 and 5% normal goat serum for 30 min at room temperature. They were then transferred to 3.0–10.0 ml PBS containing 0.3% TX-100 and 5% normal goat serum for 30 min at room temperature. The antibody utilized in this study is an affinity-purified polyclonal antibody raised against a peptide corresponding to amino acids 3–16 of the c-Fos protein. This region maps to the amino terminus of the human and mouse c-Fos protein and, therefore, does not cross-react with Fos-related antigens such as Fos B, Fra 1, or Fra 2 (27). After they were washed with PBS containing 0.1% TX-100 three times for 10 min each, the tissues were incubated with 3.0–5.0 ml of biotinylated anti-goat immunoglobulin G (1:...
Expression of c-Fos in Response to pH Changes at the VMS

Response to mCSF pH 7.4 (control). Figure 1 is a schematic representation of the ventral surface of the medulla oblongata (VMS) of the rat. Topical application of mCSF pH 7.4 (control) to the cVMS and rVMS over a 2-h period to allow sufficient time for the translation of the c-Fos protein from the induced c-fos mRNA produced a light c-Fos reaction at the sites of mCSF application. There was only scant or no c-Fos immunoreactivity throughout the rest of the neuraxis, similar to the basal levels observed in unstimulated tissues and in studies in which the animals were subjected to anesthesia and/or surgery alone (controls) (Figs. 2 and 3).

Basal levels of c-Fos were routinely detected at various brain stem sites, inclusive of the medial vestibular nucleus, ventral and dorsal cochlear nuclei, and the interpeduncular and red nuclei in the pons-midbrain.

Response to decreased mCSF pH. In response to topical application of mCSF at pH 7.2 (acidic) to the VMS over a 2-h period, the c-Fos protein reaction...
Table 1. Topical mock CSF pH 7.2 application to the cVMS and rVMS induces c-Fos protein expression at multiple brain sites

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<tr>
<th>CNS Structures</th>
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CNS, central nervous system; cVMS and rVMS, caudal and rostral ventrolateral medullary surface, respectively.

Fig. 4. Light micrograph showing the immunocytochemical Fos-positive reaction product in neurons at the AP (top left), the caudal VMS (cVMS; bottom right), in the vicinity of nXII rootlets as they exit the brain stem (top right), and the A1 vasomotor area (bottom left) in response to stimulation of the VMS with mCSF pH 7.2 (acidic). Top right: D, dorsal.
product was identified in several brain stem sites and in the hypothalamus.

At the ventral brain stem, the c-Fos reaction product was seen in the vicinity of the inferior olives (IO) bilaterally, in the lateral reticular nucleus (LRN) unilaterally, and in the parvocellular region of the LRN bilaterally (Table 1). c-Fos-positive cells were also detected in known vasoactive sites such as the noradrenaline-containing A1 vasodepressor region of the caudal ventrolateral medulla (Fig. 4). Small c-Fos-positive bipolar and multipolar neurons were observed embedded within or just beneath the marginal glia (MG) at the VMS and bilaterally just ventrolateral to the pyramids (Figs. 4 and 5). A few scattered c-Fos-positive cells were also noted within the raphé pallidus (RP) and magnus (RM) (Fig. 5, Table 1). When the decreased mCSF pH solutions were confined to the rostral brain stem chemosensitive area (rVMS), no c-Fos-positive cells were noted in the vicinity of the IO. However, c-Fos immunoreactivity was noted in the vicinity of the facial nucleus, the nucleus of the trapezoid body (NTZ), as well as the lateral and medial superior olives and the nucleus paragigantocellularis lateralis (PGCL) (Figs. 6 and 7 and Table 1). At the pontomedullary border, c-Fos immunoreactivity was detectable in the NTZ, in the ventral pontine nuclei, and in the rostral perioliary region. An intriguing cluster of c-Fos-positive cells also exists at the ventral pontine surface (VPS) in contiguity with the cells detected at rVMS levels.

At dorsal regions, immunoreactivity of c-Fos was also detected within the area postrema (AP) and the ventrolateral portion of the NTS throughout its rostrocaudal extent (Figs. 4 and 7). c-Fos reaction product was also noted bilaterally in the locus coerules (LC), nuclei parabrabialis lateralis (PBL), and Kölliker-Fuse (Fig. 7). There was also a slight response in the cerebellum in the lateral dentate and interpositus nuclei. At midbrain regions, light staining was noted at the dorsal aspects of the superior and inferior colliculi. At the level of the hypothalamus, c-Fos-positive cells were detected in the dorsomedial, lateral, and periventricular nuclei (Fig. 8). c-Fos-positive cells were also strongly expressed in the supraoptic nucleus (Figs. 8 and 9). At cortical levels, lightly staining c-Fos-positive cells were demonstrated in the piriform cortex. Taken together, these studies demonstrate that decreased pH of mCSF applied to the VMS induces c-Fos expression in a widespread network of nuclei within the brain stem and hypothalamus (Table 1).

In general, acidic stimulation of either the cVMS or the rVMS (Table 1) induced bilateral expression of c-Fos in cells at the cVMS, rVMS, and caudal VPS. This would indicate the possibility of reciprocal connections occurring between these superficial brain stem sites. cVMS stimulation induced consistent c-Fos responses at the A1 vasodepressor area, the AP, and the ventrolateral IO (Fig. 7, Table 1). rVMS activation, however, induced c-Fos immunoreactivity in the A5 area, RP, RM, cranial nerve VII, and at rostral medullary regions that appeared to be insensitive to cVMS stimulation. An intriguing response to rVMS stimulation occurred in the caudal RP that did not occur in response to cVMS stimulation. It is known that connections exist between the rostral ventrolateral medulla (RVLM) and the caudal RP; therefore, this response could have been due to descending projections from rostral regions. A finding difficult to interpret was that rVMS stimulation induced a more extensive c-Fos response at the cervical spinal cord than did cVMS stimulation. Whereas at the spinal cord level cVMS stimulation induced c-Fos responsiveness at dorsal horn cells only, rVMS stimulation additionally caused the production of c-Fos protein in cells located around the central canal (Fig. 7).

**DISCUSSION**

The exclusive localization of pH-sensitive sensing elements at the VMS that modulate respiratory activity has been a controversial issue, which has been recently compound by reports that multiple sites of respiratory chemosensitivity exist throughout the brain stem (24, 29). In an attempt to glean information on the neuroanatomic network involved in responses to brain stem CSF pH changes, determination of the
distribution of c-Fos protein expression in the rat brain was investigated in response to stimulation of the known respiratory pH-sensitive regions (19, 20, 22, 35) at the VMS with acidic mCSF (pH 7.2). The proto-oncogene c-fos and its concomitant protein are transiently, rapidly, and polysynaptically expressed within the cell nucleus in response to cell activation by a variety of stimuli. Expression of the c-Fos protein in response to CSF pH changes is, therefore, assumed to identify sites of cell activation that might contain the central respiratory chemoreceptor elements as well as neuronal elements synaptically coupled to them.

One should not, however, overlook the limitations of the c-Fos technique (7). Traditionally, 2-deoxyglucose (2-DG) uptake has been utilized as a marker of increased metabolic activity within the CNS; however, mismatches between 2-DG uptake and c-Fos activation do occur in some brain regions. These mismatches are suggested to occur because 2-DG can detect axonal and dendritic activity, whereas c-Fos stains the nucleus and is assumed, therefore, only to detect somatic changes. Additionally, some brain regions demonstrate a lack of c-Fos responsiveness, regardless of the stimulus paradigm employed. Therefore, negative results cannot be absolutely interpreted to indicate that a particular structure has not been activated. Another concern of the c-Fos technique is that basal c-Fos expression may sometimes mask changes related to the activation of specific pathways. Positive results must also be care-
fully dissected out, because activity, handling, and stress of the animal, such as can occur during surgery, may affect c-Fos levels. To avoid confounding the data with nonspecific stimuli, we did not monitor respiration or blood pressure because VMS-modulated effects on these parameters have been well characterized (19, 22). Conversely, one must ensure that the stimulus utilized was potent enough to induce a c-Fos response. It has been recognized that neurons require strong stimulation to generate c-Fos activation and that anesthetics such as ketamine and morphine can suppress c-Fos induction. The most critical aspect of c-Fos biochemistry is the time course of c-Fos activation and decay, which varies with the brain region, the type of inducing agent, and the route of application of the agent (7, 23). Additionally, it must be noted that some neurons increase c-Fos staining without an increase in firing, whereas other neurons do not increase c-Fos staining, despite an increase in firing rate (23). Careful control experiments should also be conducted to ensure that one can separate basal- from stimulus-induced c-Fos activation. We have, therefore, conducted rigorous control experiments, which included anesthesia, surgery, and neutral mCSF application controls, and determined that our experimental stimulus, acidic mCSF (pH 7.2), was able to induce c-Fos activation above basal levels in several brain regions.

**Responses at Brain Stem Sites to Low-CSF pH**

In this study, topical application of mCSF pH 7.4 (control) to the VMS produced little or no c-Fos immunoreactivity in the brain stem or throughout the rest of the neuraxis (Figs. 2 and 3). Topical application of mCSF pH 7.2 to the VMS induced a consistent and clearly identifiable distribution of c-Fos immunoreactivity throughout the rat brain. The significance of
these findings becomes evident when one reflects on the known afferent and efferent connections and functions of a number of these sites.

**Ventral brain stem cell groups.** c-Fos responsiveness to low pH at the VMS was observed in neurons within the MG ventrolateral to the pyramids and within the superficial regions of the RP. This finding corresponds well with the report of Sato et al. (29), who described cells along the VMS that were c-Fos responsive to 1 h of hypercapnia and that reside ventral to the LRN and the nucleus PGCL. These superficial cell groups in the rat are reminiscent of the type I neurons in the cat (19) and the arcuate nucleus in the human (11), which have been implicated in sudden infant death syndrome (11, 17) and Ondine’s curse (primary alveolar hypoventilation syndrome) (12).

In our laboratory, we have also detected neuronal cells by light and electron microscopy at the caudal chemosensitive area (area L) of cats within the walls of branches of the basilar artery that penetrate the ventral brain stem surface deep into the neuropil. Scheibel et al. (30) described similar specialized neurovascular relationships in various subnuclei of the raphé complex and suggested that this neurovascular system might subserve either a neurosecretor, chemosensor, mechanoreceptor, or vasomotor function. The existence of type I cells and neurovascular elements, as well as the unique properties of the MG in the caudal chemosensitive zone, have been confirmed by electron microscopy (35) and have been referred to as surface neuropil by Filiano et al. (11).

The relationship of the nucleus RP, arcuate nucleus (in cat and human), and VMS cells is an intimate one, whereby the topographic proximity and the developmental, chemical, and functional similarities argue for a common function. These regions appear to be ontogenically derived from neuronal precursors, which migrate ventromedially across the medulla from the rhombic lip (9). They subsequently differentiate into the medullary arcuate nucleus, inferior olivary complex, griseum pontis, and the serotonergic cells of the nucleus RP and nucleus PGCL. Regardless of their nomenclature, this nuclear group appears to be functionally and topographically associated with central pH-sensitive chemosensory elements at the cVMS.

The existence of neurons, as opposed to glia, in the most superficial aspects of the ventral medulla has been questioned for several years (32). In 1964, Dahlström and Fuxe (4) described serotonergic neurons in the medulla oblongata that were localized to the
raphe nuclei and in sites adjacent to the pyramids and that projected to the spinal cord. The region lateral to the pyramids and ventral to the IO was designated as the B3 region. Hökfelt et al. (15) observed dopamine decarboxylase, substance P, and serotonin [5-hydroxytryptamine (5-HT)] immunoreactivity in raphe nuclei and in the superficial cells of the arcuate nucleus located medial (ventral to the RP), ventral, and lateral to the pyramidal tract in the so-called “subpyramidal” or “arcuate” regions of the medulla. Ljungdahl et al. (18) found that substance P-containing neurons within the medulla had a similar distribution pattern to that of 5-HT neurons and that they also projected to the spinal cord. Thor and Helke (34) demonstrated that cells in the nucleus reticularis pontis and at the ventral surface of the pyramids had a high intensity of 5-HT immunofluorescence and they projected to the NTS. Many substance P immunofluorescent neurons located lateral to the pyramids and along the VMS also projected to the NTS (36), and double-labeling of cells with 5-HT and substance P (34) was detected among the arcuate fibers lateral to the pyramids, in the region of the paraventricular nucleus of Hökfelt et al. (15). In the caudal medulla, double-labeling of cells coincided with the location of the nucleus intrafasciculairis hypoglossi. Sasek and Helke (28) have also demonstrated superficial medullary enkephalineric neurons that project to the intermediolateral cell column (IML). However, electron microscopic analysis of serotonergic cells at the VMS of the rat (13) demonstrated that these cells have the usual characteristics of neurons with observable synapses on somatic and dendritic surfaces. We have, therefore, adopted the term PPR, as described by Sasek and Helke (28), to designate all superficially located, medullary neurons in the vicinity of the pyramids, inclusive of the B3 region, the paralivary region, nucleus intrafasciculairis hypoglossi, arcuate nucleus, and the traditional VMS.

In this study, c-Fos-positive cells were also identified ventral to the facial motor nucleus and in a region functionally defined as the RTN, which has extensive efferent projections to both the dorsal and ventral (33) respiratory groups. In the rat, however, the RVLM appears to contain the rostral chemosensitive zone described in the cat, which is sensitive to the acid-base changes in the CSF. The RTN could, therefore, serve as the anatomic substrate for respiratory chemosensitivity at the RVLM in the rat. At the level of the RVLM, c-Fos reaction product was also identified in neurons of the pre-Bötzinger complex [which is considered to be the rostral component of the ventral respiratory group (33)], the NTZ, ventral tegmental pontine nuclei, the rostral periolivary region, and cerebellar nuclei and within the RP and RM. Intriguingly, Richerson (26), utilizing perforated patch-clamp recordings in rat medullary slices, has shown that neurons in the rostral raphe demonstrate CO2 sensitivity and similar electrophysiological properties as previously described chemoreceptor elements.

c-Fos immunoreactivity was detected in the A1 noradrenaline area, which contains sympathoinhibitory neurons and appears to act via inhibitory connections to the RVLM or C1 area. The A1 vasodepressor site has no spinal projection (21) but does project to the NTS (34), the parabrachial complex, and the hypothalamus. Expression of c-Fos was also noted at the caudal midline raphe complex, which is a major source of descending input to the IML (21) that projects via sympathetic premotor neurons to the adrenal medulla and sympathetic ganglia. The RP and possibly raphe obscurus innervate the IML (21) and project to catecholaminergic neurons in the RVLM, implicating the caudal raphe in influencing sympathetic cells via a relay station in the RVLM. Thus detection of c-Fos immunoreactivity in the A1 area and in the caudal raphe nuclei (raphe obscurus and RM) may indicate that neuronal connectivity exists between cells at the VMS respiratory chemosensitive areas and structures involved in the regulation of blood pressure and cardiovascular activity. However, it is difficult to distinguish c-Fos activation because of VMS-stimulated blood pressure changes from that which is due to generalized stress-induced blood pressure changes. Similarly, this paradigm does not permit the precise identification of neurons that are sympathetically coupled within a CNS network.
It should be noted that there was a lack of c-Fos activation in several respiratory output neuronal groups, such as the nucleus ambiguus, nucleus paramedialis, and the hypoglossal and phrenic nuclei. There may, therefore, be regions that are responsive to VMS stimulation but do not increase their c-Fos staining. Conversely, there was an increase in c-Fos staining in regions without an obvious link to cardiorespiratory control mechanisms, such as the IO and superior olives, the facial motor nucleus, and the cervical dorsal horn, which serves to further complicate the interpretation of the data. However, the VMS pH-sensitive region may represent a site of central coinervation, where one common receptor element might impinge on both cardiorespiratory and other presently unknown autonomic control mechanisms.

**Dorsal cell groups.** The NTS is the primary site of termination of cardiovascular and peripheral respiratory afferent fibers. It appears to receive projections from the rostral and caudal (21) ventrolateral brainstem and is densely innervated by catecholaminergic fibers (4), which are believed to arise from A1 and C1 catecholaminergic cell groups within the VLM. c-Fos was expressed within the NTS throughout its rostrocaudal extent and appeared to be predominantly localized to the ventrolateral portion of the nucleus. The ventrolateral NTS is known to be the repository of lung afferents.

In response to decreased CSF pH, c-Fos immunoreactivity was identified in AP neurons, which are known to project to brain regions involved in cardiovascular regulation. The AP also receives afferent input from the periventricular hypothalamic nucleus, the PBL nucleus, the mediodorsal NTS, and the vagus nerve (32). AP projection targets include mediodorsal NTS, the PBL, C1 area, dorsal motor nucleus of the vagus, and the nucleus ambiguus (32), which may be activated in response to CSF pH changes. c-Fos-positive cells were identified in the LC in response to decreased CSF pH at the cVMS. LC or the A6 group of noradrenergic neurons may be involved in determining the level of vigilance within an animal (1) and is considered to be a site of convergence of multiple afferent inputs. At the dorsal pons, moderately stained c-Fos-positive cells were consistently noted in the nuclei PBL, parabrachialis medialis, and Kölliker-Fuse. These nuclear groups are presumed to send descending inhibitory projections to inspiratory neurons within the respiratory centers. These observations suggest the possible involvement of multiple regions of the brain stem in the responses to CSF pH changes.

**Hypothalamus.** Indeed, the identification of c-Fos immunoreactivity in the supraoptic, periventricular, and other hypothalamic nuclei appears to indicate a linkage between brain stem pH chemosensitive regions and regions involved in the regulation of water flux and possibly the antidiuretic hormone, as well as other autonomic and hypophyseotropic hormonal functions via the norepinephrine-containing monoaminergic system that projects from the LC and lateral tegmental systems throughout the spinal cord, brain stem, and hypothalamus. Sympathoexcitatory neurons scattered throughout the lateral and posterior hypothalamus project to the RVLM (5).

**Summary**

Focal, topical application of an acidic stimulus, mCSF (pH 7.2) to the VMS, both caudal (in the region of the hypoglossal rootlets) and rostral (in the region of the facial nucleus and exit of the abducens nerve), induces a nearly identical c-Fos activation pattern in the brain stem and hypothalamus of rats. The VMS or PPR is apparently the repository of several neurotransmitters and neuromodulators or their receptors and also projects to and receives projections from several CNS regions involved in autonomic control functions. Intriguingly, this study also indicates that there is a differential sensitivity between the cVMS and rVMS to an acidic stimulus, in that some structures activated by caudal stimulation are not c-Fos responsive with rostral stimulation and vice versa. Previous work and the present study, therefore, suggest that the VMS, and possibly the VPS, are potentially critical sites, within a wide and complex network of pH- and CO2-sensitive brain regions, in the central chemosensory control of respiration and other autonomic functions.

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**REFERENCES**


