Changes in c-Fos Expression Induced by Noxious Stimulation in the Trigeminal Spinal Nucleus Caudalis and C1 Spinal Neurons of Rats after Hyperbaric Exposure

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Summary. The present study aims to test the hypothesis that hyperbaric exposure inhibits nociceptive processing in the trigeminal spinal nucleus caudalis and C1 spinal neurons. We investigated the c-Fos-like immunoreactivity of the brainstem and upper cervical spinal cord (C1 region) following an injection of mustard oil (15 µl of 20%) into the nasal mucosa of pentobarbital anesthetized rats after exposure to hyperbaric (2-atmospheres, 1 h) and normobaric pressures. After the hyperbaric exposure, the mean number of Fos-immunoreactive neurons in the ipsilateral laminae I–II and III–IV of the trigeminal spinal nucleus caudalis were significantly lower than those in the normobaric condition. Similarly, the mean number of c-Fos positive neurons in the superficial layer (I–II) of the ipsilateral C1 segment were significantly reduced as compared with that in the normobaric condition. When treated with the vehicle alone, no significant difference was detected in the numbers of c-Fos positive neurons in the trigeminal spinal nucleus caudalis and C1 regions between hyperbaric and normobaric conditions. These results suggest that hyperbaric exposure may attenuate nociceptive signals from the area innervated by the trigeminal nerves at the level of both the trigeminal spinal nucleus caudalis and C1 dorsal horn.

In the acute trigeminal pain model produced by the application of mustard oil to the nasal mucosa in the rat, c-Fos positive neurons were found in the trigeminal spinal nucleus caudalis (Anton et al., 1991). By immunostaining of the c-Fos, we have recently shown that the C1-C2 dorsal horn neurons process the nociceptive information from the nasal mucosa as well as other areas innervated by trigeminal nerves, and that ethmoidal nerves may contribute to the exclusive conveyance of nociceptive information (Takeda et al., 1998).

On the other hand, a wide range of environmental conditions has been shown to modulate sensitivity to noxious stimuli (Costa and Mee, 1974). Inhibition of nociception during environmental stimuli has been expressed as either termed stress-induced analgesia or environmental-induced analgesia (Rodgers and Randall, 1988). Environmentally induced analgesia occurs in animals following exposure to various stimuli such as inescapable tail or foot shock, immobilization and forced cold water swimming (Rodgers and Randall, 1988). Concerning atmospheric conditions, it has been reported that hyperbaric exposure alters the pattern of pain-related behavior in the formalin test (Berge et al., 1991). Furthermore, exposure to hyperbaric oxygen is effective in relieving the pain attacks of patients suffering from cluster headaches (Miller, 1981; Weiss, 1989; Sabato et al., 1993). These studies lead us to suggest that hyperbaric exposure may reduce the nociceptive transmission. However, little attention has been paid to the effect of atmospheric conditions on the neuronal transmission of nociceptive information from orofacial regions.

The present study, therefore, was undertaken to
test the hypothesis that hyperbaric exposure attenuates the nociceptive processing in the trigeminal spinal nucleus and C1 spinal neurons. For this, we investigated c-Fos immunoreactivity in these areas following an injection of mustard oil into the nasal mucosa of pentobarbital anesthetized rats subjected to normobaric and hyperbaric conditions.

MATERIALS AND METHODS

Experiments were conducted on 15 male adult Wistar rats (310-450 g). They were divided into 4 groups according to experimental procedures: 1) Normobaric-mustard oil group (n=3), 2) Normobaric-paraffin oil group (n=3), 3) Hyperbaric-mustard oil group (n=5), and 4) Hyperbaric-paraffin oil group (n=4). Hyperbaric exposure was performed as follows: The rats were habituated to a steel chamber for 2 h immediately before hyperbaric exposure. After habituation of the rats in the steel chamber (2 h), the pressure was increased to 2-atmospheres within 2 min, and maintained for 1 h. Oxygen partial pressure was maintained at approximately 40%, and the chamber temperature was controlled to 25-26°C. In the chamber, each rat was free to move within the rat cage. After hyperbaric exposure, a chemical stimulant or vehicle was injected into the animals anesthetized with sodium pentobarbital (45-50 mg/kg, i.p.). In order to stimulate the ethmoidal nociceptors in the

![Diagram showing the distribution of c-Fos immunoreactive neurons in the brain stem and in the C1 region. A: normobaric condition, B: hyperbaric condition. AP area postrema, NTS nucleus tractus solitarius, LRN lateral reticular nucleus, SpV and SpVc trigeminal spinal nucleus interpolaris and caudalis. Number is the distance (mm) of the frontal plane in relation to the obex. 0.0: just at the obex, +0.5: rostral to the obex, −0.5: caudal to the obex.](image-url)
nasal mucosa, 15 μl of 20% mustard oil (allylisothiocyanate) in paraffin oil was injected into the right nasal meatus by means of a blunt Hamilton syringe. Rats receiving 15 μl of pure paraffin oil into the nasal cavity were used as controls.

All the rats were deeply anesthetized with pentobarbital (45 mg/kg, i. p.), two hours after the injection of mustard oil or the vehicle, they were perfused transcardially with heparinized saline followed by 4% paraform aldehyde in 1/15 M phosphate buffer (PB) at pH 7.4. The brain stem and C1 spinal segment were removed, fixed for 2 h in the same fixative, and soaked stepwise in 10%, 20% and 30% sucrose in 1/15 M PB until block submergence. The frozen brain and spinal cord were cut into 40 μm coronal sections. Every third sections was processed by the immunohistochemical technique described in a previous report (Takeda et al., 1998). The sections were then preincubated alternatively in avidin and biotin solutions, incubated with sheep anti-c-Fos polyclonal antiserum (OA-11-824, Cambridge Research Biochemicals) at a dilution of 1:1000, incubated in an ABC solution (Vector Labs.) and treated with a DAB-nickel solution containing 0.003%
H$_2$O$_2$. The sections were then examined under a light microscope. Each neuron in which block staining of the nucleus was visualized was termed Fos-positive, regardless of the staining intensity. The locations of c-Fos positive neurons were mapped and counted in 7 sections per rat. Brain histology was checked by using the brain atlas of PAXINOS and WATSON (1986). Statistical significance was calculated by Duncan's new multiple range test. A probability of less than 0.01% was considered to be significant.

RESULTS

After exposure to the hyperbaric oxygen, in mustard oil-induced respiratory parameters (such as the frequency of sneezing and duration of apnea) no obvious change was detected compared to those seen with normobaric oxygen. Changes in inflammation parameters (e.g., mucosal swelling, reddening, and secretion) after hyperbaric exposure were somewhat less than those in the normobaric condition.

Figure 1 shows a schematic demonstration of c-Fos positive neurons of both brain stem and C1 coronal sections. In vehicle-treated rats, no notable changes in the site of the c-Fos expression were observed in either the normobaric or the hyperbaric condition. In both normobaric and hyperbaric conditions, mustard oil-induced c-Fos immunoreactivity could be found bilaterally in the nucleus tractus solitarius, the area postrema, the lateral reticular nucleus, and the C1 segment spinal cord. Clear-cut differences between the hyperbaric and normobaric conditions in the numbers of c-Fos positive cells induced by mustard oil application were detected in the ipsilateral trigeminal spinal nucleus caudalis and C1 spinal dorsal horn neurons (Figs. 1, 2).

The mean numbers of c-Fos immunoreactive neurons per section in the laminae I–II and III–IV of the ipsilateral trigeminal spinal nucleus caudalis were significantly lower than those in rats with normobaric exposure ($p<0.01$) (Fig. 3A). Similarly, in the C1 segment of the spinal cord, the mean numbers of c-Fos positive neurons per section in the superficial layer (Laminae I–II) were significantly smaller than those in the normobaric conditions ($p<0.01$) (Fig. 3B). No significant difference was observed between vehicle-treated normobaric and hyperbaric rats in mean numbers of c-Fos immunoreactive neurons per section.

Fig. 3. Comparison of the number of mustard oil-induced Fos-immunoreactive neurons in the trigeminal spinal nucleus caudalis (SpVc, A) and C1 spinal dorsal horn (B) between normobaric and hyperbaric conditions. Significant differences between normobaric and hyperbaric conditions. *: $p<0.01$. 

![Graph A](image1)

![Graph B](image2)
DISCUSSION

In this study, the number of c-Fos immunoreactive neurons in the trigeminal spinal nucleus caudalis and C1 spinal cord in hyperbaric oxygen-treated rats largely decreased compared with those in the normobaric oxygen-treated rats. A hyperbaric condition is known to affect various physiological parameters including autonomic function (TARASIUK and GROSSMAN, 1991; TREMMELLEN et al., 1993). For example, in the absence of sensory input, exposure to hyperbaric pressure depresses central respiratory activity in the isolated brain stem-spinal cord preparation (TARASIUK and GROSSMAN, 1991). In this study, hyperbaric exposure did not cause any significant changes in the c-Fos expression in the brain stem nuclei, including the nucleus tractus solitarius and the area postrema that regulates the function of the autonomic nervous system. Moreover, no significant difference was found between the number of c-Fos positive cells in vehicle-treated rats in hyperbaric and normobaric conditions. However, if more appropriate stimulation (a higher pressure) is applied, one can expect significant changes in the c-Fos expression to occur in the two nuclei. Thus, it is possible that the expression of c-Fos may not directly reflect the change in the autonomic function.

Dorsal horn neurons in the C1 segment of the rat spinal cord play a significant role in the nociceptive transmission from trigeminal nerves (TAKEDA et al., 1989; MATSUMOTO et al., 1999). A recent report has shown that the rostro-caudal distribution of c-Fos expression neurons in the C1 spinal cord of the cat becomes more prominent by the increasing electrical stimulation intensity of tooth pulp and by applying mustard oil to the tooth pulp (IWATA et al., 1989). In the present study, after hyperbaric exposure, the mean numbers of c-Fos positive neurons in the superficial and deep layers of the trigeminal spinal nucleus caudalis were significantly lower than those seen in the normobaric condition. In the C1 spinal neurons, the mean number of c-Fos positive neurons in the superficial layer was only significantly lower than that in the control. The difference between the trigeminal spinal nucleus caudalis and C1 segment on the location of c-Fos positive cells may be primarily due to the numbers of projection terminals of the trigeminal nerve (RUGGIERO et al., 1981; PFALLER and ARVIDSSON, 1988). Based on the fact that c-Fos positive neurons in the superficial laminae were strongly attenuated, it can be inferred that the hyperbaric condition would inhibit the transmission of nociceptive information from the nasal mucosa as well as from other areas innervated by trigeminal nerves.

The mechanism by which c-Fos positive neurons following noxious chemical stimulation of nasal mucosa are inhibited in rats after exposure to hyperbaric pressure is unclear. Several influences on the appearance of this inhibitory action can be considered. First, it has been shown that descending inhibitory systems inhibit the noxious stimulation-evoked expression of the c-Fos protein (JONES and LIGHT, 1990; GOGAS et al., 1991; ZHANG et al., 1994). Lesions of the dorsolateral funiculus increase formalin-induced c-Fos expression (ZHANG et al., 1994). These dorsolateral funiculus lesions can block the suppression of c-Fos expression produced by the intracerebroventricular administration of enkephalin in rats after formalin injection to the hindpaw (GOGAS et al., 1991). Moreover, a pharmacological blockade of descending inhibitory system decreases hyperbaric analgesia, in which pain sensitivity can be measured in a test used for electrical stimulation of the tail (IGNATOV et al., 1992). These lines of evidence suggest that the inhibitory effects of the hyperbaric exposure on the c-Fos expression in both the trigeminal spinal nucleus caudalis and C1 spinal neurons may be mainly due to the activation of the descending pain control system. Second, it has been reported that the hyperbaric exposure induces vasoconstriction (MILLER, 1981), facilitates the diffusion of oxygen into the various tissues (FISHER et al., 1988), and stimulates seroton synthesis in the central nervous system (COSTA and MEEK, 1974). These events, occurring after hyperbaric exposure, may promote local inhibitory changes in the c-Fos expression seen in the present study.

Finally, a partial increase in the pressure of inert gases exerts narcotic and anesthetic effects in both human divers and experimental animals (BENNET, 1993). It is also known that inert gases binding the modulatory site of protein receptors act as an allosteric regulator (ABRAHIM et al., 1998). Since the c-Fos protein expression is also known to be affected by various anesthetic drugs (TAKAYAMA et al., 1994), it is possible that attenuation of the c-Fos expression may be partly involved in the anesthetic effect of inert gases, but this will have to be clarified by further studies.

REFERENCES


