Recordings From Brain Stem Neurons Responding to Chemical Stimulation of the Subarachnoid Space

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Ebersberger, A., M. Ringkamp, P. W. Reeh, and H. O. Handwerker. Recordings from brain stem neurons responding to chemical stimulation of the subarachnoid space. J. Neurophysiol. 77: 3122–3133, 1997. The subarachnoid space at the base of the skull was perfused continuously with artificial cerebrospinal fluid in anesthetized rats. A combination of inflammatory mediators consisting of histamine, bradykinin, serotonin, and prostaglandin E2 (10^{-5} M) at pH of 6.1 was introduced into the flow for defined periods to stimulate meningeal primary afferents. Secondary neurons in the caudal nucleus of the trigeminal brain stem were searched by electrical stimulation of the cornea. Of the units receiving oligosynaptic input from the cornea, 44% were excited by stimulation of the meninges with inflammatory mediators. Most of these units had small receptive fields including cornea and the periorbital region, and their responsiveness was restricted to stimuli of noxious intensity. Three types of responses to stimulation of the meninges with algogenic agents were encountered: responses that did not outlast the stimulus period, responses outlasting the stimulus period for several minutes, and oscillating response patterns containing periods of enhanced and suppressed activity. The response pattern of a unit was reproducible, however, upon repetitive stimulation at 20-min intervals; the response magnitude showed tachyphylaxis upon stimulus repetition. The preparation presented mimics pathophysiological states normally accompanied by headache, e.g., subarachnoidal bleeding. Responsiveness of neurons in the caudal nucleus of the trigeminal brain stem to inflammatory mediators may play a role in the generation and maintenance of headache, e.g., migraine.

INTRODUCTION

Since 1940, sensory nerve fibers supplying the dura mater encephali have been thought to be involved in the generation of headache (Penfield and McNaughton 1940; Ray and Wolff 1940). In the last two decades, it has been shown that the dura is innervated densely by thin myelinated and unmyelinated nerve fibers (Edvinsson and Uddman 1981; Keller and Marfurt 1991). Most of these fibers are probably afferent because they contain substance P (SP) and calcitonin gene related peptide (CGRP) known to occur exclusively in primary afferents (Edvinsson et al. 1989; Fang 1961; Keller and Marfurt 1991; Matsuyama et al. 1986; Yamamoto et al. 1983). The majority of the ganglion cells that innervate the dura of cat and rat are located in the ophthalmic division of the trigeminal ganglion (Keller et al. 1985; Mayberg et al. 1984; O’Connor and Van Der Kooy 1986; Steiger et al. 1982).

Though these meningeal afferents are thought to provide input for the generation of headaches, little is known about the mode of their activation. The dreadful headaches associated with subarachnoid hemorrhage probably are caused by bloodborne substances that come into contact with the meninges. In the case of headaches typical for meningitis, there are presumably inflammatory mediators involved (part of which, again, are bloodborne). According to a new hypothesis, the migraine, a more common form of headache, is related to a neurogenic inflammation in the meninges (Goadsby 1993; Moskowitz and MacFarlane 1993). Indeed, it has been demonstrated that neuropeptides are released in the meninges when trigeminal nerve fibers are stimulated electrically (Goadsby et al. 1988; Zagami et al. 1989); depletion of neuropeptides can be observed by the application of capsaicin to cerebral vessels (Duckels and Buck 1982; Janssen et al. 1989). Furthermore, SP, Neuropeptide Y and serotonin-like immunostaining were reduced substantially after stimulating the dura by application of autologous arterial blood (Keller et al. 1993). After release, neuropeptides may activate or sensitize meningeal nociceptive afferents (Holzer 1988). During migraine attacks, release of CGRP and SP could be measured in the jugular vein (Goadsby et al. 1988, 1990), adding evidence to the neurogenic inflammation hypothesis. A link between peripheral neuronal activity and central excitation is indicated by the observation that SP is released in the trigeminal brain stem nuclear complex after chemical stimulation of the meninges (Schaible et al. 1996) and by the measurement of an enhanced level of excitatory amino acids, such as glutamate, in the cerebrospinal fluid of migraine patients during the attack (Martinez et al. 1993).

Thus all lines of evidence lead to the conclusion that stimulation of the meninges with chemical mediators provides an important mechanism in the generation of various forms of headaches. Substances with algogenic activity may be released either in the course of the pathophysiological process itself or indirectly as a consequence of the release of neuropeptides that induce plasma extravasation.

It is known from clinical observations that subarachnoidal rather than epidural stimuli cause intense headache (Dalessio 1989) and that the meninges at the base of the skull are particularly sensitive. However, the few studies on sensitivity of meningeal afferents applied chemical stimuli either into the sagittal sinus or to the epidural surface (Dostrovsky et al. 1991; Lambert et al. 1991; Zagami and Lambert 1990, 1991). Therefore the aim of the study was to describe an experimental method for applying chemical stimuli to the subarachnoidal space by superfusion of the basal meninges in the anesthetized rat. We assessed input from the dura in response to application of inflammatory mediators by single unit recordings of secondary neurons in the subnucleus caudalis, the principle relay of orofacial pain and headache (Ses-
NEURONS RESPONDING TO CHEMICAL MENINGEAL STIMULATION

Methods

Animal preparation

Experiments were performed on adult male Wistar rats (280–450 g). The animals were anesthetized with thiopental (120 mg/kg body wt ip). After scopolamine injection (50 mg/kg sc.), the trachea as well as the femoral vein and artery were cannulated. Subsequently, the animals were mounted in a stereotactic frame with the head fixed in horizontal position. The animals were ventilated artificially. The end-tidal CO$_2$ was adjusted between 4.5 and 5.5%. The rats were paralyzed with pancuronium (initial dose 3.7 mg/kg iv; supplemental doses 1 mg·kg$^{-1}$·h$^{-1}$ iv). This dosage of pancuronium was sufficient to prevent movements due to spontaneous respiration but allowed control of blink and withdrawal reflexes. The intra-arterial blood pressure and the heart rate (ECG) were monitored continuously. Anesthesia was monitored by regularly testing the corneal blink reflex and the responses to paw pinch. Supplemental doses of thiopental were given to maintain areflexia (25 mg/kg ip). The body temperature was kept at a constant level of $\sim 38^\circ$C by a rectal thermometer and a feedback-controlled infrared lamp. The eyes were covered with an ointment (Bepanthen, Roche) to prevent noxious input due to drying of the cornea.

The skullpan was exposed, and a hole (0.8 mm diam) was drilled that led into the cleft between the underlying frontal lobe and the left olfactory bulb (4 mm rostral to Bregma, 2.5 mm lateral from midline). To get access to the subarachnoid space, a catheterer (0.8 mm diam) was inserted and pushed cautiously forward (4 mm) to the base of the skull. Finally, the catheter was glued with acrylic to the surface of the skull. Thereafter the head of the animal was ventroflexed (90°), and, using standard surgical procedures, the cerebellum and the brain stem were exposed. Before starting the perfusion, $\sim$80% of the cerebellum was aspirated to ensure an unimpeded outflow of the perfusion fluid. The lesion gave sight to the bottom of the fourth ventricle. The subarachnoid space then was perfused with artificial cerebrospinal fluid (ACSF) consisting of (in mEq$^{-1}$) 150 Na$^+$, 3 K$^+$, 2.5 Ca$^{2+}$, 1.2 Mg$^{2+}$, 132 Cl$^-$, and 25 HCO$_3^-$ plus 3.7 mM glucose and 6 mM urea, pH 7.4 (Nozaki et al. 1992) via the frontal catheter. A continuous flow of 4.5 ml/h was maintained by a syringe pump (Fig. 1A). Chemical stimuli were injected as a bolus into the continuous flow of ACSF. After forming a perceptive field of each neuron was drawn on a schematic figure of the rat’s face. Convergent maxillary input was assessed by noxious stimuli applied to the upper incisor teeth. To test for neuronal activity due to mechanical stimulation alone, a foam rubber pellet was at first slightly pressed to the incisors (O). This was repeated after cooling the pellet with dichlorodifluoromethane gas (common vitality test in dentistry; P: noxious cold).

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As a control, a bolus of 100 $\mu$l ACSF was channeled into the continuous perfusion flow of ACSF 10 min before the chemical stimulation. To chemically stimulate the meninges, a mixture of inflammatory mediators (1M) consisting of histamine, bradykinin, serotonin, and prostaglandin $E_2$ each 10$^{-3}$ M, pH 6.1 in ACSF was used (see Kessler et al. 1992). To visualize the in- and outflow and to measure the duration of the chemical stimulation, Evans Blue was added to the control (ACSF) and to the stimulus. With the volume (100 $\mu$l) and the flow rate (4.5 ml/h) used, the passage through the skull lasted $\approx$ 120 s. Twenty minutes later (in 3 cases, 15 min later), the stimulus was repeated. In some cases, an identical stimulus was applied 20 min after the second one. At maximum, five chemical stimuli were applied to one animal at intervals of $\approx$ 20 min. In each animal, only one chemically excitable cell was recorded. To control the extension of the chemically stimulated area (stained area) at the base of the skull, the brain was removed in 20 animals at the end of the experiment. Additionally, the stained meninges were peeled from the ventral part of the brain and as far as possible from the inside of the skull.

Data analysis

Action potentials were discriminated off-line by their shape and size using the SPIKE/SPIDI software package (Forster and Handwerker 1990). Responses to the stimuli of the standard protocol for characterization of the neuron (see above) were qualitatively analyzed in neurons that also responded to chemical stimulation. Neuronal responses to chemical stimulation of the meninges were treated as follows: the 2-min period before stimulation to 12 min after stimulus application was subdivided in 10-s bins. The spontaneous activity of a neuron was quantified as the mean number of action potentials $\pm$ SD from the 10-s bins of the prestimulus period. A response to a stimulus was defined as positive when the activity of the unit exceeded the spontaneous activity at least two times the standard deviation during the first 4 min after stimulus application. The first 10-s bin meeting this criterion was taken as start of the response. The same criterion was used to define the end of a response. In figures showing averages from several units (Fig. 5, A and B), the spontaneous activity has been subtracted.
A period of 12 min after the start of a response was used to analyze the time course of the response.

RESULTS

Neurons were searched throughout the whole rostro-caudal extension of the subnucleus caudalis of the trigeminal brain stem complex in a depth of 0–2,800 μm from the surface of the brain. Recordings from 117 units responding to the electrical search stimulus at the cornea were stable enough for characterization with regard to the type of input they received, to their mechanoreceptive fields in the face, and to responsiveness upon cold stimulation of the incisor teeth. Those units were tested subsequently by stimulation of the meninges with inflammatory mediators (see METHODS). Fifty-one of the neurons (43.6%) responded to chemical stimulation of the meninges.

Characterization of the neurons

LATENCIES OF THE RESPONSES TO ELECTRICAL STIMULATION OF THE CORNEA. In neurons receiving convergent input from meninges and cornea, the latencies between the stimulus and the first action potential ranged from 7 to 22 ms (in 1 case 47 ms; Fig. 2). Assuming mono- or oligosynaptic input, these latencies suggest Aβ- and/or C-fiber input from the cornea because the estimated conduction distance was ~2 cm (mean conduction velocity 1.74 m/s). To ensure a mono- or oligosynaptic input, only units with constant latencies to repeated stimulation were included in the sample. Examples of spikes evoked by electrical corneal stimulation are shown in several specimen figures (Figs. 3B and 4A).

RECEPTIVE FIELDS OF CHEMICALLY EXCITABLE NEURONS. Only 12 of 51 units (23.5%) driven by chemical stimulation of the subarachnoid space responded to weak mechanical stimuli like air puffs (in Figs. 3 and 4, B = blowing) or stroking the hairs in the receptive field (RF) with a soft brush (H = hair). In contrast, all 51 units responded to high intensity (noxious) mechanical stimuli (PR = prick; PI = pinch; E = pinch at the eyelid).

Most cutaneous RFs were small and entirely restricted to the innervation territory of the ophthalmic division of the trigeminal nerve, typically including the upper and/or lower eyelid. Because electrical stimulation of the cornea was used as a search stimulus, RFs often included the cornea when tested mechanically; RFs of 31 (out of 51) neurons were confined to this region. In some cases, however, additional RFs were found in the innervation area of the maxillary trigeminal division, including the upper incisor teeth (in 13 of 51 units), the nostril (8 of 51 units), or the snout (6 of 51 units).

Table 1 shows a summary of these data. For each of the three most common combinations of receptive properties, an example of the complete characterization protocol is provided in Fig. 3. Figure 3A shows a unit with a mechanical receptive field at the eyelid and the cornea that responded...
FIG. 3. Examples for 3 most common response patterns of the neurones in nc caudalis submitted to a standard characterization protocol. A: neuron with a mechanical receptive field at eyelid and cornea (a), action potential after electrical stimulation of cornea (b). In c, responses to mechanical stimulation of face and to tooth pulp stimulation are shown (see METHODS). Unit responded only to high intensity stimuli at eyelid (E, PE) and cornea (C). B: unit with a receptive field at eyelid and skin above eye (a), activity after electrical stimulation of cornea (b). Unit showed a low frequent spontaneous activity throughout characterization procedure. It responded to low-intensity stimuli like striking hairs of face with a fine brush (H) but also to high-intensity stimuli like pricking (PR) skin above eye and pricking and pinching at eyelid (E, PE). C: neuron with a receptive field only at upper eyelid (a), activity after electrical stimulation of cornea (b). Unit was excited only by high-threshold stimulus at eyelid (E, PE). Abbreviations: B, blowing to face; H, striking hairs of face with a fine brush; PR, pricking to facial skin; PI, pinching to facial skin; E, pinching to eyelid; PE, pricking to eyelid; C, tapping to cornea; O, applying a foam pellet (room temperature) to upper incisor teeth; P, noxious cold (applying a cooled foam pellet to upper incisor teeth).
FIG. 4. Specimen of a neuron responding to chemical stimulation of meninges with a mixture of inflammatory mediators.  
A: response to electrical stimulation of cornea (7 V, 0.4 ms). B: mechanical receptive field at upper eyelid. C: responses to stimulation of facial receptive field. D: response to chemical stimulation of meninges with inflammatory mediators (IM, pH 6.1). Response shows a brief onset and a relatively fast decline. ACSF, application of artificial cerebrospinal fluid as a control; bars, period of stimulus application (~120 s).

to pinching and pricking of the eyelid (E, PE) and to lightly tapping at the stimulation electrode attached to the cornea (C). There was no response to weak mechanical stimuli (B, H) and to noxious cold at the toothpulp (P). In contrast, the unit shown in Fig. 3B was also excited by low-intensity mechanical stimulation of the skin above the eye. In Fig. 3C, a unit only responding to pricking and pinching of the eyelid is shown. Six units were of this type.

Only 1 out of 13 neurons with a facial receptive field restricted to the maxillary region responded to chemical stimulation of the meninges, whereas 50 out of 100 neurons including the ophthalmic region were activated by stimulation of the meninges.

Thirty seven of the units with input from the chemically stimulated meninges (72%) were spontaneously active (>0.5 s⁻¹). Twenty two of them had larger than average RFs, including skin areas outside the periorbital region in the ophthalmic (n = 5) or maxillary region (n = 17). In contrast, eight meningeal units with very small receptive fields (cornea, eyelid, or eyelid and cornea) showed no spontaneous activity (<0.5 s⁻¹).
The last column shows the numbers of neurons that showed a specific combination of receptive areas as indicated in the line left to the number. Numbers in the last line of the table show how many neurons responded to stimulation of a specific area. +, response to the applied stimulus; −, no response to the applied stimulus in the indicated area (stimulation procedure described in METHODS).

Localization of the neurons

Two clusters of neurons receiving convergent input from the meninges and the cornea were encountered, one in the very rostral and the other one in the caudal part of the subnucleus caudalis. The rostral cluster was found in a region extending in the rostro-caudal direction between 0–300 μm from obex and 2,000–2,800 μm lateral from the midline. The caudal cluster was located between 2,000–2,300 μm caudal from the obex and 1,600–2,500 μm mediolateral from midline. The units in both clusters were found at a mean depth of 1,245 ± 532 μm from the dorsal surface of the brain stem, indicating a localization primarily in the ventral part of the subnucleus caudalis.

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LOCALIZATION OF THE AREA STIMULATED. In all experiments, Evans Blue was added either to the solutions containing the stimulating agents or to the bolus of ACSF (used for control in this study). The extension of the stained part of the basal meninges was examined in 20 animals postmortem. In all of these animals, at least the ipsilateral and medial parts of the dura overlying the circle of Willis were stained (Fig. 1B). In some cases (n = 9), the perfusion fluid was spreading to the contralateral site. The frontal meninges at the olfactory bulbs and the parts of the dura covering the convexities of the brain remained unstained. No staining was found in the tissue underlying the meninges.

RESPONSES TO PERFUSION OF THE SUBARACHNOID SPACE. Superfusion of the meninges with ACSF did not excite any of the units within an observation period of 10 min. In four neurons, slightly enhanced activity exceeding spontaneous activity by one standard deviation was observed. However, 44% of the 117 neurons tested showed a clear response to superfusion of the meninges with ACSF containing a combination of IM consisting of histamine, bradykinin, serotonin, and prostaglandin E2 at pH 6.1. Figure 4 shows a neuronal response with a quick onset and a relatively fast decline upon passage of inflammatory mediators within ~120 s. In this neuron, a second application of the stimulus at an interval of 20 min led to a similar response.

RESPONSE PATTERNS. According to their different response patterns, the neurons were categorized in three groups: units in which firing did not outlast the stimulus (120 s), units showing responses that considerably outlasted the stimulus duration of 120 s, with a slow recovery to their initial level of spontaneous activity before the next stimulus was applied 20 min later, and units exhibiting bi- or polyphasic responses (definition see METHODS). Figure 5 shows histograms of the mean responses of the units of the first and second group. In this figure, spontaneous activity has been subtracted. In mean longer lasting responses showed higher response rates when shorter lasting ones.

The units belonging to the third class with long lasting bi- or polyphasic response pattern are displayed in Fig. 6. The responses of most of these units were biphasic with an initial excitation followed by a depression of spike activity. Both parts of the response were apparently not related to the duration of the stimulus. Four out of the 16 neurons of this class showed oscillating excitation after chemical stimulation and were therefore regarded to respond polyphasically. Duration and frequency of the oscillating activity varied from cell to cell.

Neurons with different chemical response patterns also could be distinguished by their different spontaneous activity. In the first class, only 22% of the neurons (showing a response not outlasting the stimulus duration) were spontaneously active (>0.5 s⁻¹) in the interval 2 min before the stimulus. More frequently, neurons of the other two classes with the outlasting response patterns showed spontaneous discharges exceeding one spike/2 s before the application of inflammatory mediators (49 and 44% of class 2 and 3 units, respectively).

TACHYPHYLAXIS. Independent of the response pattern, the units usually were excited less by repetition of the chemical stimulus. Although the first stimulus elicited, on average, 15.43 ± 6.93 impulses/10 s during the following 10 min, the second stimulus evoked only 4.49 ± 9.18 impulses/10 s. In some cases, a third stimulus was applied that yielded similar responses as the second one. Figure 7 demonstrates the tachyphylaxis in a unit to which three stimuli were applied at intervals of 15 min.

DISCUSSION

This study demonstrates that a proportion of neurons of the trigeminal subnucleus caudalis responds to chemical stimulation of meningeal structures with a combination of inflammatory mediators. Dural afferents were stimulated by chemicals that had direct access to the inner site of the dura. This form of application resulted in a rapid onset of
responses of brain stem neurons comparable with that observed in primary nociceptive afferents studied by superfusion with a similar cocktail of mediators in a skin-nerve preparation (Kessler et al. 1992).

Functional characteristics of neurons receiving input from the meninges

SEARCH STRATEGY. For searching, electrical stimulation of the cornea was used rather than chemical stimulation of the meninges, because meningeal nerve terminals may have become sensitized during the search procedure or, alternatively, tachyphylaxis after repeated chemical stimulation might have concealed responses. Electrical stimulation of the subarachnoid space was avoided to prevent the trigeminal ganglia and nerve bundles from direct activation by current spread. By the chosen searching procedure (electrical cornea stimulation), a subpopulation of neurons has been selected that has convergent input from the eye and from the meninges. The total proportion of brain stem neurons excited by chemical stimulation of the meninges may therefore be underestimated. However, similar populations of neurons were found when electrical search stimuli were applied to the dura (see below, RECEPTIVE FIELDS).

LATENCIES. The long activation latencies after electrical stimulation of the cornea indicate input from slowly conducting myelinated or unmyelinated afferents. This was expected, because the cornea mainly is innervated by thin afferent nerve fibers (Belmonte et al. 1991). Presumably the input from the meninges also is transmitted by thin nerve fibers, because electrical stimulation of the dura in cat revealed an exclusive Aδ input from the dura to the caudal trigeminal nucleus (Davis and Dostrovsky 1986, 1988; Dostrovsky et al. 1991; Strassman et al. 1986). These data also are supported by recordings in the subnucleus caudalis and in the trigeminal ganglion in rat where the response latencies to electrical stimuli reflect input from Aδ-fibers (Messlinger et al. 1995; Strassman et al. 1995).

RECEPTIVE FIELDS. Ninety percent (46/51) of the neurons activated by chemical stimulation of the meninges had facial receptive fields restricted to the innervation territory of the ophthalmic division of the trigeminal nerve. This observation is consistent with receptive field locations reported for neurons searched by direct stimulation of the dura after craniotomy (cat: Davis and Dostrovsky 1986, 1988; Dostrovsky et al. 1991; Lambert et al. 1991; Messlinger et al. 1995; Strassman et al. 1986; human: Wirth and Van Buren 1971). Clinical experience also shows that headache often is localized to some extend behind or above the eye and, vice versa, ophthalmic diseases like glaucoma often are accompanied by severe headache. Additional receptive areas were observed in the maxillary trigeminal division in 20 neurons. As described by Sessle et al. (1986), caudalis neurons may receive input from up to six different areas of receptive fields.

FIG. 5. Averaged responses (minus spontaneous activity) to inflammatory mediators; 2 populations of units with different response patterns were found. Each column represents a 10-s bin width. Bar indicates application period of stimulus (~120 s). A: mean response of neurons that were activated for longer than stimulus duration. B: mean response of neurons, activity of which did not outlast stimulus duration.
including ophthalmic, maxillary and mandibular cutaneous regions, intranasal and intraoral structures, and deep tissue like the masseter muscle.

Because extracranial and intracranial structures such as meninges are not likely to be innervated via collaterals of branching primary afferents (Borges and Moskowitz 1983; McMahon et al. 1985), the complex receptive fields of the neurons result from convergent inputs onto brain stem neurons from different primary afferents innervating extra- and intracranial structures.

The notion that units in the nucleus caudalis mediating meningeal input are getting mainly nociceptor input from the body surface is corroborated by the finding that the observed receptive fields had a high activation threshold and were relatively small, suggesting that they had not been much altered in their size by sensitizing processes (compare with Hu et al. 1992; Sessle et al. 1986). The activation threshold and the receptive field size of the neurons were not tested at the end of the experimental procedure. Units with larger receptive fields and additional input from low-threshold mechanoreceptors often exhibited spontaneous activity as also described for unit populations with input from deep somatic tissue and/or skin in brain stem and spinal cord (Gilette et al. 1993; Hu 1990).

**Recording sites**

In this study, neurons with convergent inflow from the basal meninges, the cornea and/or the ophthalmic region were found in the very rostral and caudal part of the subnucleus caudalis of the trigeminal brain stem complex. Because the trigeminal subnuclei are spindle shaped and overlap in their transition zones, it cannot be excluded that some of the recorded neurons belonged to subnucleus interpolaris or the cervical spinal cord (C1). There are no data from electrophysiological studies in the rat concerning the localization of brain stem neurons with meningeal input. In accordance with studies in the cat, we found neurons with meningeal input in the deeper laminae of the caudal trigeminal nucleus (Davis and Dostrovsky 1986, 1988; Strassman et al. 1993).

**Fig. 6.** Activities of neurons (minus spontaneous activity) in NC caudalis that responded bi- or polyphasically to stimulation with IM. ● ● ●, beginning and end of application (duration 120 s). Each column represents a 10-s bin.
FIG. 7. Repeated responses of a neuron that exemplifies tachyphylaxis to chemical stimulation of meninges with IM. Different line plots show response of unit to first, second, and third stimulus given to animal at a 15-min interval for a duration of about 2 min.

Sites of chemical stimulation

The staining of the passage way of the test solutions through the subarachnoid space showed that at least the ipsilateral part of the circle of Willis was exposed to the chemical stimulus. The innervation of the meninges is particularly dense around the large vessels at the base of the skull (Edvinsson et al., 1981, 1987; Suzuki et al., 1989). Presumed sensory fibers containing SP and CGRP form a dense network close to dural and pial vessels (Edvinsson et al., 1989; Fang, 1989; Keller and Marfurt, 1991; Matsuyama et al., 1986; Yamamoto et al., 1983). Single beaded fibers run along the vessel wall or terminate in the connective tissue between blood vessels (Messleringer et al., 1993). Most of these fibers run within the ophthalmic division of the trigeminal nerve to the trigeminal ganglion (Keller et al., 1985; Mayberg et al., 1984; O’Connor and Van Der Kooy, 1986; Suzuki et al., 1989b; Steiger and Meakin, 1984). Very few of them project to the brain stem via the maxillary and mandibular division of the trigeminal nerve.

It needs to be considered that the chemical stimulation procedure could have activated the brain stem neurons rather directly than synaptically by the activation of meningeal afferent fibers. This is unlikely for the following reasons. First, no staining could be seen on the surface of the brain stem after peeling off the stained meninges from the underlying tissue. Short stimulus duration and continuous flow of the fluid may have prevented a passing of the algogenic molecules through the meninges to the underlying tissue. Second, only 1 out of 13 neurons with a facial receptive field restricted to the maxillary region responded to chemical stimulation of the meninges whereas, 50 out of 100 neurons including the ophthalmic region were activated by stimulation of the meninges. Thus the neurons were activated by the chemical stimulus only in a region with substantial input from the meninges (via the ophthalmic branch). It is most likely, therefore, that the responses of the brain stem neurons were evoked synaptically by activation of meningeal afferents rather than by direct stimulation.

Chemical stimulus

Inflammatory mediators as used in the present study are presumably involved in the headache accompanying meningitis or subarachnoid hemorrhage where they can be assumed to be bloodborne. Additionally, in the last decade, migraine headaches often have been considered to be due to a neurogenic inflammation of the meninges (Goadsby, 1993; Lauritzen, 1994; Moskowitz and MacFarlane, 1993) by plasma extravasation. This again would lead to exposure of meningeal nociceptors to inflammatory mediators. The present experiments have shown an intense activation of second order neurons in the subnucleus caudalis by inflammatory mediators, indicating a dominant role of these nociceptive brain stem neurons in headache. The inflammatory mediators applied in this study to the meninges are substances that indeed...
can be found under pathophysiological conditions as during neurogenic inflammation. Serotonin, for example, can be released from perivascular sympathetic nerve endings (Stanley et al. 1993), from platelets and mast cells after release of neuropeptides by activation of sensory afferents (Dimitriadou et al. 1992; Kraeuter Kops et al. 1990; Öhlén et al. 1989). All of the substances are able to excite or sensitize primary afferents (Kessler et al. 1992; Lang et al. 1990) as well as postsynaptic neurons, resulting in lowered stimulus threshold, expansion of the receptive fields, and increased spontaneous activity (Woolf 1994). Thus inflammatory mediators released in meningeal structures (see Goadsby et al. 1990; Juul et al. 1995; Moskowitz et al. 1983; Saito et al. 1987; Zagami et al. 1989) should be able to cause headache by activation of trigeminal brain stem neurons.

Responses to chemical stimulation of the subarachnoid space

Excitation of neurons in the nucleus caudalis of the cat was observed by Dostrovsky et al. (1991) after application of bradykinin into the sagittal sinus. Epidural application of capsaicin and bradykinin also caused activation of thalamic neurons (Lambert et al. 1991; Zagami and Lambert 1990, 1991). In accordance, the present data show that 44% of the nucleus caudalis neurons recruited by electrical stimulation of the cornea also responded vigorously to stimulation of the subarachnoid space with a mixture of inflammatory mediators.

In an in vitro skin-nerve preparation, a similar mixture activated 85% of the polymodal primary nociceptive afferents (Kessler et al. 1992). In this test solution, the excitatory action seemed to be mainly due to the presence of bradykinin (Lang et al. 1990) and a low pH (Steen et al. 1995). In contrast, histamine, and serotonin are described to play a minor role in excitation. At present similar studies on primary meningeal afferents are lacking.

Response patterns. After stimulation with acidic inflammatory mediators, three different response types were found: responses outlasting or not outlasting the stimulus duration and responses of bi- or polyphasic character. It is unclear to what extent these response patterns reflect the peripheral input and/or central modifications. Single polymodal receptors in the skin, in vitro, showed a large variability in the response patterns after stimulation with bradykinin (Lang et al. 1990), whereas responses to pH 6.1 tended to last as long as the stimulus was present, showing little or no adaptation (Steen et al. 1992). If meningeal afferents behave similarly, a test solution with low pH should cause sustained responses as observed in all of the three response patterns. However, the perfusion of the subarachnoid space has the disadvantage that the duration and temporal position of a stimulus depended on the exact location of the receptive field of a neuron. In spite of this, most of the neurons with the stimulus-outlasting response pattern definitely fired for longer than algesic substances could have been present in the subarachnoid space (> 10 min). A similar time course has rarely been found in primary afferents of the skin. Although nothing is known about afferents of the subarachnoid space in this respect, it may be that the long duration of the response probably was generated, at least in part, by the second order neurons. Such a phenomenon of a much slower decline of the discharges in secondary dorsal horn neurons as compared with primary afferent fibers has been observed after heat stimuli (Handwerker et al. 1975).

TACHYPHYLAXIS. Induction of tachyphylaxis was observed regularly upon repeated application of inflammatory mediators. In the skin, a lack of tachyphylaxis to inflammatory mediators was described when the pH of the solution was lowered to 6.1 (Steen et al. 1995). However, tachyphylaxis was well present in an in vitro preparation of visceral afferents from the rabbit pleura upon repeated stimulation with the mixture of inflammatory mediators at pH 6.1 (Weidner et al. 1996), which might be a better model for dural afferents. In addition, low pH was probably not safely maintained in our preparation throughout the stimulus duration due to neutralization by the buffering capacity of the subarachnoid space. When bradykinin alone or a mixture of inflammatory mediators were applied to primary cutaneous afferents at neutral pH pronounced tachyphylaxis was observed regularly (Kessler et al. 1992; Lang et al. 1990). Therefore it is very likely, that the tachyphylaxis observed in the present study was due to altered primary afferent activity. As shown in Figs. 7 and 4D, the shape of a response was not much altered by repeating the stimulus. Therefore it is not likely that cells with a rapid decrease in response (not outlasting the stimulus duration) can be explained by tachyphylaxis.

In conclusion, an experimental model is described that allows to study neuronal mechanisms involved in nociceptive processes in the meninges. In the present experiments, we have demonstrated that the application of inflammatory mediators is a powerful stimulation to excite neurons in the trigeminal nuclear complex. The receptive fields of these neurons are located mainly in regions supplied by the ophthalmic branch of the trigeminal nerve and reflect locations of pain often observed in headache patients. Because headaches have been associated with the occurrence of pathophysiological processes in the meninges (e.g., neurogenic inflammation), we believe that the experimental model described here is suitable to investigate mechanisms of certain forms of headaches.

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