Rhinology, 36, 168–172, 1998

Responses to nasal irritation obtained from the human nasal mucosa*

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SUMMARY

Responses to chemical irritation can be obtained from the human respiratory mucosa in response to stimulation with gaseous CO_2; these negative mucosal potentials (NMPs) are thought to be summated receptor potentials from chemosensitive nociceptors. The present study aimed to investigate the relation of this response to both stimulus concentration and perceived intensity. A total of 29 healthy volunteers participated. Maximum negative amplitudes occurred 1.1 s after stimulus onset. The negativity exhibited a higher coefficient of correlation to intensity estimates of the painful sensations (r =.65) than to the stimulus concentration (r =.46); it appeared at the same time when the subjects' tracking of the painful sensations reached its maximum amplitude. These findings suggest that the NMP is suited for the investigation of peripheral nociceptive events in man.

Key words: Mucosal potential, nociception, chemosensory evoked potential, trigeminal chemoreception, pain measurement, peripheral response

INTRODUCTION

As early as 1981 Kobal (Kobal, 1981) recorded responses to painful chemical stimulation directly from the surface of the human nasal mucosa. These responses were clearly related to the subjects' intensity estimates. Kobal (Kobal, 1985) also demonstrated that this negative mucosal potential (NMP) was not related to changes in skin resistance, could be extinguished by means of a local anaesthetic, and changed after systemic administration of the opioid pentazocine indicating the presence of opioid receptors in the peripheral nociceptive system (see Stein and Yassouridis, 1997). In addition, Thürauf et al. (Thürauf et al., 1991) demonstrated a decrease of the NMP's amplitude after having administered capsaicin both locally and systemically to experimental animals. Moreover, using guanethidine they demonstrated the independence of the NMP from autonomic reflexes. Hence, it can be assumed that the NMP is a specific nociceptive event.

The present study aimed to re-investigate the relation of the NMP to both stimulus concentration and perceived intensity. To use this technique in the investigation of peripheral nociception the present study appeared to be necessary as Kobal (Kobal, 1985) had only presented results from four subjects.

MATERIAL AND METHODS

Twenty-nine young healthy volunteers (17 male and 12 female subjects, between 18 and 37 years of age; mean age 25 years) participated in the experiment after they had provided written informed consent. The study was performed in accordance to the Declaration of Helsinki / Hong Kong.

Study design

Prior to the actual experiment subjects were acquainted with the experimental conditions and procedures. All participants were trained in a breathing technique (velopharyngeal closure), whereby respiratory flow inside the nasal cavity during stimulation is avoided (Kobal and Hummel, 1989).

Two sessions were performed on separate days, each of which lasted approximately 30 min. During this time, 30 painful CO_2 stimuli were delivered to the left nostril all of which were clearly above threshold. Subjects were comfortably seated in an accoustically shielded, ventilated room. White noise, of approximately 50 dB SPL, was used to mask switching clicks of the stimulator. In order to stabilise vigilance, subjects were requested to perform a simple task on a video screen during stimulus presentation and the interstimulus intervals. Using a

* Received for publication November 25, 1997; accepted June 26, 1998

joystick they had to keep a small square inside a larger one which moved unpredictably (Kobal et al., 1990).

Stimulation of the nasal mucosa

For nasal stimulation, a specially devised stimulation technique was employed which allowed delivery of the chemical stimulants without altering the mechanical or thermal conditions at the stimulated mucosa. This monomodal chemical stimulation is achieved by mixing pulses of the stimulants in a constantly flowing air stream with controlled temperature and humidity (36.5° C; 80% relative humidity). The air stream was led into the nasal cavity by way of a teflon tubing (6 cm length, 4 mm outer diameter). The total flow rate was 140 ml/sec. Stimuli had a rise time below 20 msec; for further details see (Kobal, 1985; Kobal and Hummel, 1988). Stimulus duration was 500 ms, interstimulus interval was approximately 60 s. Three concentrations of carbon dioxide (45, 52, and 59% v/v CO_2) were delivered in a randomised sequence.

Negative mucosal potential (NMP)

The NMP was recorded from the left nasal septum via a tubular electrode filled with Ringer-agar (1%) that contained a silver-chlorided silver wire (impedance 2-8 kΩ at 1 kHz in 0.9% NaCl). After the electrode had been placed by either the experimenter or by the subject, it was firmly held in place by means of an adjustable clip which was fixed to a frame of lensless glasses (Hummel et al., 1996). For reference a silver-chlorided silver disc electrode (3.2 mm^2) was attached contralaterally close to the medial eye angle. Responses were recorded by means of DC-amplifiers (Toennies, Germany; bandpass DC-30 Hz). After analogue-to-digital conversion (sampling rate 125 Hz; CED1401, UK) records of 16,384 ms were stored on disks of an IBM-compatible computer (SIGAVG program package, Cambridge Electronic Devices, UK). Each epoch included a prestimulus period of 230 ms which served as baseline for the base-to-peak measures of amplitudes. Before averaging, the data were screened for artifacts produced by eye-blinks or muscular activity (e.g., swallowing); only artifact-free records were analysed.

The latencies (in relation to stimulus onset), base-to-peak amplitudes, and peak-to-peak amplitudes were measured separately for the three concentrations ((Kobal, 1985); see also Fig. 2 for peak identification). The first positive peak occurred approximately 420 ms after stimulus onset (P4), the first negativity after 1,140 ms (N11), and the second positivity after 4,000 ms (P40).

Estimates of painful intensities

Following presentation of a painful stimulus the subjects estimated its perceived intensity in relation to a standard stimulus applied at the beginning of the session (52% v/v CO_2); the standard's intensity was defined as 100 estimation units (EU). Subjects rated intensities by means of a computerised visual analogue scale displayed. For statistical evaluation, the subject's ratings for each session were averaged separately for each stimulus concentration.

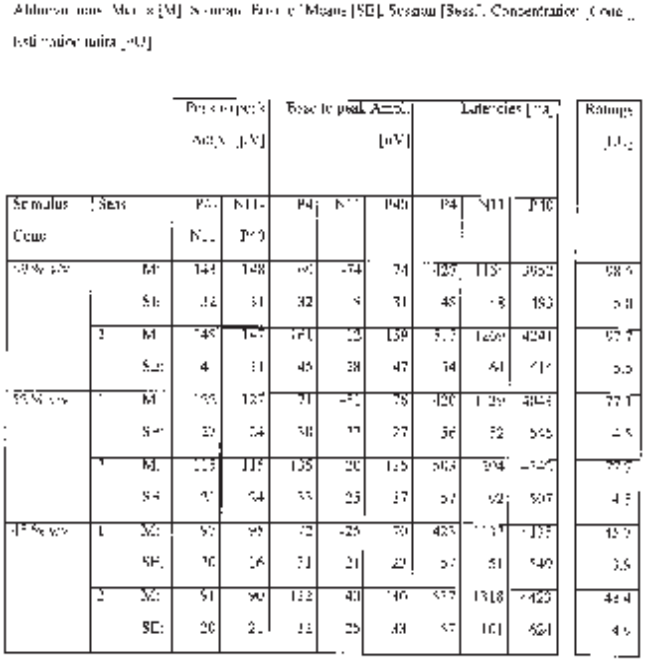
Statistical analyses

SPSS 6.1.3 for Windows™ was employed for statistical evaluation. NMP data of any subject were included in the analysis only if the responses to all three stimulus concentrations could be evaluated. For example, if an NMP to the highest CO_2 concentration was contaminated by eye-blinks, the responses to the medium and low concentration were not used for further evaluation. Data of both sessions were submitted to analysis of variance (MANOVA, repeated measurement design, with "stimulus concentration" as within-subject-factor). To investigate concentration related linear changes of the responses, trend-analyses were employed only when the MANOVA yielded significant differences ($p<0.05$). To get an impression how the NMP was related to either the stimulus' concentration or the intensity estimates, two correlations were computed separately for each subject. After Fisher's Z-transformation the coefficients of correlation were averaged across all subjects. In addition, correlations (Pearson) were computed separately for each stimulus concentration between data obtained during the two sessions.

RESULTS

Table 1 presents descriptive statistics of the investigated parameters; Table 2 gives a summary of the statistical analysis of the data.

Table 1: Descriptive statistics of investigated parameters (1st session: n=19; 2nd session: n=18)



Mucosal potentials: Responses from the nasal mucosa (Figure 1) could be obtained in all 29 subjects. However, in the first session data of only 19 subjects, and, in the second session of 18 subjects could be used for statistical analysis. This was due to the criterion that only those subjects should be included into the final analysis in whom it was possible to obtain artifact-free responses to all three stimulus concentrations; in addition to contamination of the NMP with artifacts, in a number of sub-

Table 2: Summary of Statistical Analyses.

			MANOVA df	MANOVA F-ratio	MANOVA p-value	Trend r-value	Trend p-value
Intensity Estimates							
Estimates		Sess. 1	5/52	114.72	0.001	12.05	0.001
		Sess. 2	5/52	79.43	0.001	9.16	0.001
NMP							
Peak to Peak Amplitudes	P1N1	Sess. 1	3/62	5.15	0.01	3.24	0.01
		Sess. 2	3/62	4.12	0.05	2.56	0.05
	N11P40	Sess. 1	3/62	6.39	0.01	2.58	0.05
		Sess. 2	3/62	9.91	0.001	3.21	0.01
Base to Peak Amplitudes	P4	Sess. 1	3/62	0.23	n.s.	-	-
		Sess. 2	3/62	2.33	n.s.	-	-
	N11	Sess. 1	3/62	8.18	0.05	2.80	0.05
		Sess. 2	3/62	4.66	0.05	2.81	0.05
	P40	Sess. 1	3/62	0.16	n.s.	-	-
		Sess. 2	3/62	2.23	n.s.	-	-
Latencies	P4	Sess. 1	3/62	0.10	n.s.	-	-
		Sess. 2	3/62	0.39	n.s.	2.71	0.05
	N11	Sess. 1	3/62	1.31	n.s.	-	-
		Sess. 2	3/62	0.56	n.s.	-	-
	P40	Sess. 1	3/62	0.36	n.s.	-	-
		Sess. 2	3/62	3.16	n.s.	-	-

Table 3: Coefficients of Correlations.
Coefficients were made between parameters of the NMP and both, the stimulus' concentration (Conc), and the subjects' intensity estimates (Ets). The table presents average coefficients of correlations

	Peak to peak Amplitudes		Base-to-peak Amplitudes			Latencies		
	P4N11	N11P40	P4	N11	P40	P4	N11	P40
NMP - Conc.	+.62	+.51	+.07	+.49	+.12	+.19	+.01	+.04
NMP - Est.	+.67	+.49	+.05	+.55	+.04	+.33	+.08	+.04

jects recordings from the epithelium were unstable, i.e., baseline drifts occurred during the course of the session which made reliable peak identification impossible. Both peak-to-peak amplitudes, P4N11 and N11P40, increased with rising concentrations of CO_2 ($p<0.05$) (Figure 2). However, when investigating base-to-peak measures, a relationship to the stimulus concentration was only found for the N11 amplitude ($p<0.05$). Latencies did not significantly change in relation to the stimulus concentration, with the exception of the P4 latency during the second session. Coefficients of correlation between NMP amplitudes obtained in the first and the second session were generally low; highest coefficients were obtained for amplitude N11 (59% v/v CO_2: r=.33, 52% v/v CO_2: r=.35, 45% v/v CO_2: r=.25). In contrast, N11 peak latencies exhibited a much higher correlation between test and re-test (59% v/v CO_2: r=.79, 52% v/v CO_2: r=.52, 45% v/v CO_2: r=.42).

Estimates of painful intensities

Estimates of stimulus intensity exhibited a linear increase with rising stimulus concentration ($p<0.001$) (Figure 2). Coefficients of correlation test and retest were good (59% v/v CO_2: r=.81, 52 % v/v CO_2: r=.65, 45% v/v CO_2: r=.81).

Correlations between NMP and both, stimulus concentration, and intensity estimates: When compared to latencies amplitudes of the NMP correlated best with both intensity ratings and the stimulus concentration (Table 3). Coefficients were generally larger when amplitudes were correlated with the subjects' estimates compared to the correlations between stimulus concentration and NMP amplitudes. Relatively large coefficients (≥49) were found for the peak to peak amplitudes P4N11, and N11P40. In contrast, coefficients for base-to-peak amplitudes P4 and P40 were smaller than .14; only the N11 base-to-peak amplitude yielded coefficients as high as .65; this indicated that the results obtained for the peak-to-peak amplitudes were mainly due to the N11 base-to-peak amplitude.

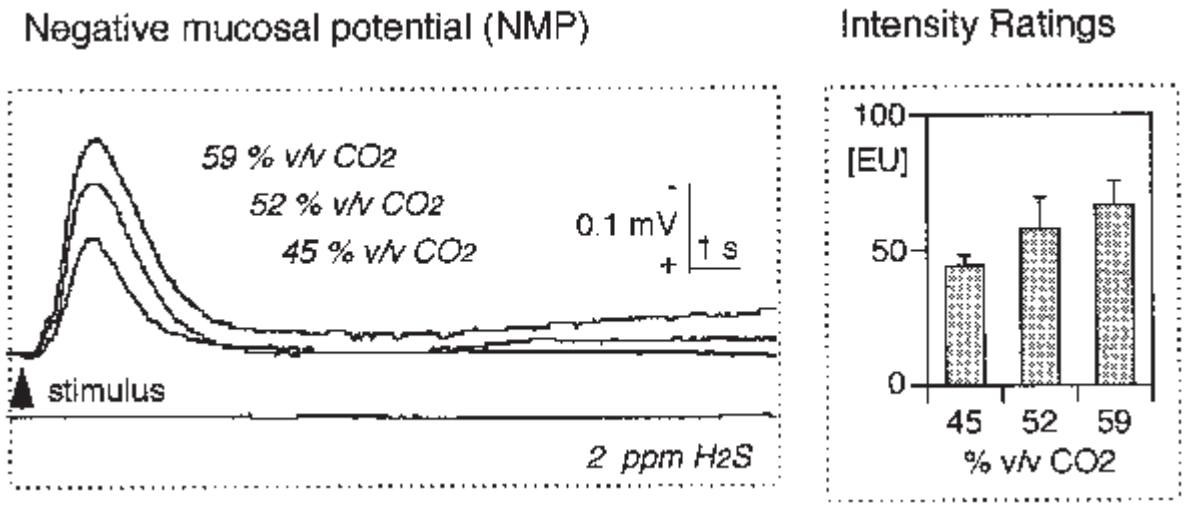


Figure 1: Examples of recordings obtained in a single subject. Left: Negative mucosal potential (NMP). Right: Mean intensity estimates (+ standard errors; EU: estimation units; 0 EU = "no painful sensation", 100 EU = "painful intensity of standard stimulus"). Both, NMP amplitudes and intensity ratings increased as a function of stimulus concentration. In this subject recordings were also made in response to 2 ppm H_2S stimuli of 500 ms duration shown at the bottom left; although these olfactory stimuli produced a very intense, unpleasant olfactory sensation, they did not elicit a response from the respiratory epithelium.

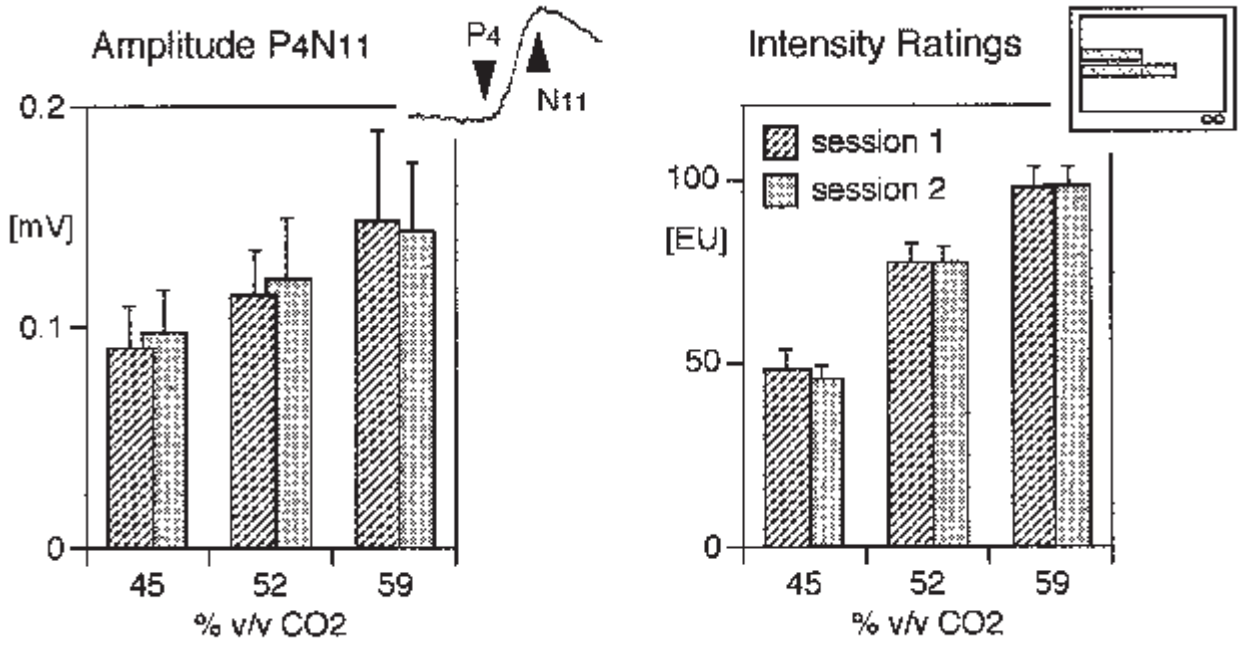


Figure 2: Means (+ standard errors) of NMP amplitudes P4N11 (in mV) and intensity ratings (in estimation units, EU) in response to 3 concentrations of CO_2 (59, 52, and 45% v/v), separately for the first (hatched bars; n=19) and the second session (dotted bars; n=18). The left insert is a schematic drawing of an NMP; peaks P4 and N11 are identified by arrows. The right insert shows the computerised visual analogue scale; subjects rated intensity in comparison to a standard applied at the beginning of the session. Both, NMP amplitudes and intensity ratings increased as a function of the stimulus concentration.

DISCUSSION

The experiment clearly established that the NMP is related to perceived irritation. It is important to note that the amplitude of the negative peak N11 exhibited a better correlation to the subjects' estimates than to changes in stimulus concentration. This may be interpreted in terms of adaptive changes at a peripheral level (Handwerker et al., 1987; Hummel et al., 1996; Van Hees and Gybels, 1972) that are reflected to a similar degree in both the electrophysiological and the psychophysical response [compare (Chen et al., 1979)]. It clearly emphasizes that the NMP is suited for the investigation of peripheral nociceptive events in man. In addition, the experiment confirmed previous studies indicating that CO_2 evokes painful sensations in a concentration-related manner (Kobal and Hummel, 1989) as reflected in the subjects' intensity estimates.

However, it has also been found, that responses to all 3 stimulus concentrations could only be analysed in approximately 65% of the trials. While this yield restricts the range of routine clinical applications (Gracely, 1989) it is easily remedied by a more careful selection of subjects. Considering the non-invasive character of this peripheral measure of nociception this drawback appears to be tolerable, especially in view of other techniques used to assess peripheral nociceptive events, e.g., microneurography (Torebjörk and Hallin, 1971), which may potentially lead to neural damage.

Reasons for the relatively low test-retest reliability of the response may be sought in differences of electrode placement between sessions. As it is conceivable that recordings from different sites produce different responses, future studies using endoscopical control of the mucosal recording site (compare (Hummel et al., 1996)) are expected to yield a much higher test-retest reliability of the NMP. Aside from these considerations, using a repeated measures, 3-fold cross-over design, the NMP has only recently been shown to allow the assessment of dose-related effects of the non-steroidal anti-inflammatory drug ibuprofen (Lötsch et al., 1997). Considering the major technical improvement which can be expected from the endoscopic electrode placement the NMP can be viewed as a highly promising tool for the non-invasive investigation of peripheral nociception. Recent investigations by De Wijk, Cain and Pilla-Caminha (De Wijk et al., 1998) additionally indicate that the NMP may be used to assess nociceptive thresholds. Specifically, measurement of the NMP in response to a range of suprathreshold stimuli provides data which allow estimation of thresholds based on the extrapolation of the stimulus response function. In fact, using ethanol as a trigeminal stimulant in a group of 10 healthy subjects, remarkably similar thresholds were found for psychophysical (3,500 ppm) and electrophysiological threshold measures (3,700 ppm).

It is surprising to see that the N11-negativity of the mucosal potential develops as late as 400 ms after stimulus onset, i.e., it starts around the time when the N1-peak of EEG-derived evoked potentials develops (Hummel et al., 1994), and when subjects signal the onset of painful sensations induced by CO_2 (Kobal, 1981; Kobal, 1985). It has been speculated that the onset of the negativity is obscured by the P4-positivity which is poorly related to the stimulus concentration. Although it is argued that the P4-positivity is merely an electrochemical artifact produced by stimulation with CO_2 (Müller, 1971) it can be completely extinguished by means of a local anaesthetic (Kobal, 1985)

indicating a possible physiological origin [see also (Gesteland, 1971)]. This observation raises the question of the origin of the response obtained from the nasal mucosa which is thought to be generated by chemosensory nociceptors of the trigeminal nerve (Kobal, 1985). This is supported by anatomical studies indicating that the nerve endings are arranged in an orderly way providing the electrophysiological basis for the generation of summated potentials (Cauna, 1982). Furthermore, Thürauf et al. (Thürauf et al., 1991) demonstrated the reduction of the NMP's amplitude by capsaicin in experimental animals. Their investigations also indicated the NMP's independence from the pain-related activation of the autonomic nervous system (Thürauf et al., 1993). In contrast, it has also been claimed that the response – or at least parts of it – might be associated with the activity of nociceptors in response to an axon reflex [compare (Barnes et al. 1986; Bouvet et al., 1987)] which is induced by painful stimulation with CO_2. Clearly, further experiments with a special focus on the refractory period of this phenomenon are needed to address this question.

Summarily, the present findings suggest that the NMP is suited for the investigation of peripheral nociceptive events in man. In this context it seems to be important to note that the same technique can be applied in experimental animals (Thürauf et al., 1991). The potential for parallel investigations in humans and animals appears to be one major advantage of this technique.

ACKNOWLEDGEMENTS

This research was supported by DFG grant Ko812/1-5 and by grant P01 DC 00161 from the National Institute on Deafness and Other Communication Disorders, National Institute of Health, USA.

REFERENCES

1. Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind PW (1986) Histamine is released from skin by substance P but does not act as the final vasodilator in the axon refle. Br J Pharmacol 88:741-745.
2. Bouvet JF, Delaleu JC, Holley A (1987) Does the trigeminal nerve control the activity of the olfactory receptor cells? Ann NY Acad Sci 510:187-189.
3. Cauna N (1982) Blood and nerve supply of the nasal lining. In: Proctor DF, Andersen I, eds. The nose: upper airway physiology and the atmospheric environment. Amsterdam: Elsevier, pp. 46-97.
4. Chen ACN, Chapman CR, Harkins SW (1979) Brain evoked potentials are functional correlates of induced pain in man. Pain 6:365-374.
5. de Wijk R, Cain WS, Pilla-Caminha G (1998) Human psychophysical and neurophysiological measurements on ethanol. ChemSenses (in press).
6. Gesteland RC (1971) Neural coding in olfactory receptor cells. In: Beidler L, ed. Handbook of Sensory Physiology, Chemical Senses, Olfaction, Vol. IV/1. Berlin: Springer, 132-150.
7. Gracely RH (1989) Methods of testing pain mechanisms in normal man. In: Wall PD, Melzack R, eds. Textbook of pain. Edinburgh: Churchill Livingstone, pp. 257-268.
8. Handwerker HO, Anton F, Reeh PW (1987) Discharge patterns of afferent cutaneous nerve fibers from the rat's tail during prolonged noxious mechanical stimulation. Brain Res 65:493-504.
9. Hummel T, Gruber M, Pauli E, Kobal G (1994) Event-related potentials in response to repetitive painful stimulation. Electroenceph Clin Neurophysiol 92:426-432.
10. Hummel T, Knecht M, Kobal G (1996) Peripherally obtained electrophysiological responses to olfactory stimulation in man: electro-olfactograms exhibit a smaller degree of desensitization compared with subjective intensity estimates. Brain Res 717:160-164.
11. Hummel T, Schiessl C, Wendler J, Kobal G (1996) Peripheral electrophysiological responses decrease in response to repetitive painful stimulation of the human nasal mucosa. Neurosci Letters 212:37-40.
12. Kobal G (1981) Elektrophysiologische Untersuchungen des menschlichen Geruchssinns. Stuttgart: Thieme Verlag.
13. Kobal G (1985) Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. Pain 22:151-163.
14. Kobal G, Hummel C (1988) Cerebral chemosensory evoked potentials elicited by chemical stimulation of the human olfactory and respiratory nasal mucosa. Electroenceph Clin Neurophysiol 71:241-250.
15. Kobal G, Hummel C, Nürnberg B, Brune K (1990) Effects of pentazocine and acetylsalicylic acid on pain-rating, pain-related evoked potentials and vigilance in relationship to pharmacokinetic parameters. Agents Actions 29 3/4:342-359.
16. Kobal G, Hummel T (1989) Brain responses to chemical stimulation of the trigeminal nerve in man. In: Green BG, Mason JR, Kare MR, eds. Chemical Senses, Vol.2: Irritation. New York: Marcel-Dekker, 123-139.
17. Lötsch J, Hummel T, Kraetsch HG, Kobal G (1997) The negative mucosal potential: separating central and peripheral effects of NSAIDs in man. Eur J Clin Pharmacol 52:359-364.
18. Müller W (1971) Vergleichende elektrophysiologische Untersuchungen an den Sinnesepithelien des Jakobsonschen Organs und der Nase von Amphibien (Rana), Reptilien (Lacerta) und Säugetieren (Mus). Z vergl Physiol 72:370-385.
19. Stein C, Yassouridis A (1997) Peripheral morphine analgesia. Pain 71:119-121.
20. Thürauf N, Friedel I, Hummel C, Kobal G (1991) The mucosal potential elicited by noxious chemical stimuli: is it a peripheral nociceptive even. Neuroscience Letters 128:297-300.
21. Thürauf N, Hummel T, Kettenmann B, Kobal G (1993) Nociceptive and reflexive responses recorded from the human nasal mucosa. Brain Res 629:293-299.
22. Torebjörk HE, Hallin RG (1971) Perceptual changes accompanying controlled preferential blocking of A and C fibre responses in intact human skin nerves. Exp Brain Res 16:321-332.
23. Van Hees J, Gybels J (1972) Pain related to single afferent C-fibers from human skin. Brain Res 48:397-400.

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