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Comparative analysis of human nasal mucosa by standard neurohistochemical techniques

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SUMMARY

The modified glyoxylic acid histofluorescent method (Torre and Surgeon) and Karnovsky-Roots method for acetylcholinesterase activity was applied to fresh animal and human material. Human material was obtained during ENT surgery and consisted of nasal, laryngeal and hypopharyngeal mucosa. Not only on animal, but also on human material adrenergic axons and varicosities were demonstrated around vessels in nasal mucosa, diffusely in nasal, laryngeal and hypopharyngeal mucosa and just below and intraepithelially in laryngeal mucosa. Abundant cholinergic innervation was found around vessels and glands in nasal, laryngeal and hypopharyngeal mucosa, as well as diffusely scattered in the tissue of their mucosa.

INTRODUCTION

Functional and morphological studies of nasal mucosa neurovegetative innervation have been carried out intensively in recent years (Nomura and Matsuura, 1972; Änggård and Edwall, 1974; Änggård, 1974; Wilson and Yates, 1977; Änggård, 1979; Krajina, Krmpotić and Večerina, 1979; Lundberg et al., 1980; Uddman, Malm and Sundler, 1981). Although some of the research projects relate to human material, the majority of investigations were performed on experimental animals. The results of animal experiments (usually offering the well-known advantages, such as plenty of material for analysis, reproducibility, testing of the methodology) cannot be directly applied to human physiology and pathophysiology. This statement could be reinforced by the fact that animal research results are very often controversial varying from species to species. This is particularly true in the field of nasal physiology, mainly in the field of neurovegetative nasal innervation.

Although investigation of human nasal innervation, apart from ethical, is complicated with many technical difficulties such as premedication in ENT surgery and

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lack of standardization, each analysis of human material offer new, relevant and comparable data.

Based on the classical image of neurovegetative innervation of nasal mucosa (according to Nomura and Matsuura, 1972) we presumed that in human nasal mucosa acetylcholin is released around the vessels and glands, while vasoconstruction is mediated via adrenergic fibers. Thus, we hypothesized that current neurohistochemical methods (tested mainly on animal experimental material) are also relevant methods in human nasal physiology and that they can be used as research and possibly routine laboratory procedures.

MATERIAL AND METHODS

Although the aim of this study was innervation of human nasal mucosa as revealed by some current histochemical techniques, we carried out some animal experiments to test the sensitive glyoxulic acid method for monoaminergic demonstration.

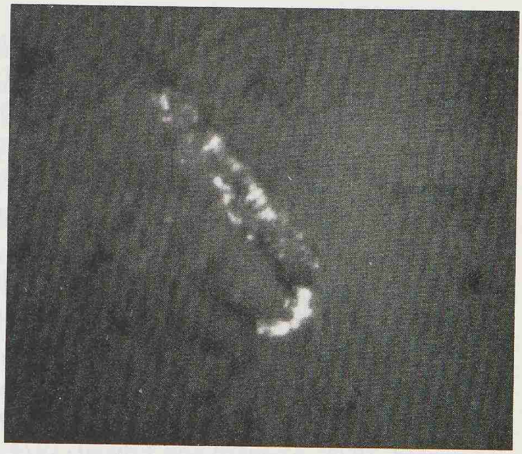
A modified approach of the glyoxylic acid condensation reaction (de la Torre and Surgeon, 1976) was utilized for visualization of biogenic amines. Acetylcholinesterase activity in the tissue was demonstrated by a modification of Karnovsky-Roots' method (1964). The specificity of acetylcholinesterase reaction was controlled by several inhibitors.

The fluorescence method with glyoxylic acid condensation was primarily tested on fresh animal material: suprarenal gland, ductus of epididymus and mesenterium of ten adult rats. Fresh human material obtained during ENT surgery subjected to the same histofluorescent procedure included: 10 specimens of nasal septum and inferior nasal turbinate, two specimens laryngeal mucosa, one of hypopharyngeal mucosa and one of soft palate (uvula). Acetylcholinergic innervation was investigated on 20 specimens of the nasal septum and inferior nasal turbinate obtained during routine nasal surgery, on five specimens of laryngeal mucosa and on one specimen of hypopharyngeal mucosa (sinus piriformis mucosa). Specimens of laryngeal and hypopharyngeal mucosa could be considered important control material for various comparative studies.

RESULTS

Monoaminergic axons and varicosities (green fluorescence phenomenon) were clearly demonstrated in the suprarenal gland, ductus of epididymis and around vessels in rat's mesenterium. Fibers and varicosities of the same characteristics in the fluorescent microscopy were identified around vessels in several specimens of human inferior turbinate (Figure 1). In human nasal mucosa (septum and concha) some other monoaminergic fibers were found, but it was impossible to determine their connection with certain histological structures. Thin monoaminergic axons were demonstrated also in the uvula and in laryngeal mucosa. In laryngeal

Figure 1. Adrenergic innervation around vascular lumen in human inferior nasal turbinate.



mucosa, just below the basal membrane and partially penetrating to the epithelium, typical monoaminergic varicosities were proved. In some specimens of the inferior turbinate and nasal septum mucosa-abundant bright yellow granular deposits were found (around vessels, glandular ducts and diffusely in tissue) which could be easily misinterpreted as specific monoaminergic innervation.

Rich cholinergic innervation was demonstrated on stretch specimens of the inferior nasal turbinate and of the ventricular and vocal fold. On serial sections of such specimens we localized acetylcholonesterase activity which could be easily recognized around vessels and glands in nasal (Figure 2) and ventricular fold mucosa.

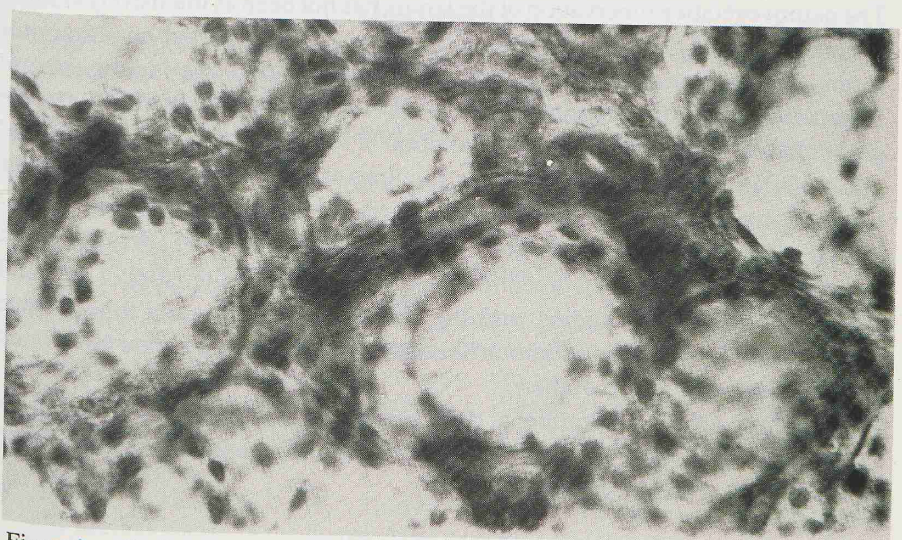


Figure 2. Cholinergic innervation of vessels and glands in human inferior nasal turbinate.

DISCUSSION

The findings of adrenergic axons and varicosities and acetylcholinesterase activity in the analyzed human and animal material proved the reliability of applied research methods. The accumulation of intensive yellow activity around some structures in human nasal mucosa could be misleading and provoke wrong conclusions about positive specific fluorescent findings. Such artefacts indicate the necessity of choosing highly precise criteria in evaluating the results of neurohistochemical research in humans.

The majority of recent publications in the field of neurovegetative innervation of nasal mucosa deal with the peptide containing nerve in nasal mucosa causing vasodilatation resistant to atropin (Änggård, 1979; Uddman et al., 1981; Lundberg et al., 1980). Modern researchers prefer functional more than classical neurohistochemical methods because they offer more complete information (Änggård, 1974; Änggård and Edwall, 1974; Wilson and Yates, 1977). Some of them tried to identify adrenergic receptors in nasal mucosa (Änggård and Edwall, 1974).

Publications about the neurohistochemistry of human nasal mucosa are relatively scarce. Nomura and Matsuura (1972) found in human nasal mucosa, rich cholinergic innervation around glands and vessels and the complete absence of adrenergic innervation around nasal glands. On the contrary, Änggård (1974) observed a sparse network of fluorescent fibers surrounding the separate acini. Our findings of adrenergic activity around vessels in human nasal mucosa and the rich cholinergic network around glands and vessels match the results of Nomura and Matsuura.

The neurovegetative innervation of the larynx has not been as intensively studied as innervation of the nasal mucosa. Shin et al. (1970) reported on vasomotor responses to laryngeal nerve stimulation. They demonstrated in dogs that stimulation of vagus and the recurrent laryngeal nerve resulted in 30% increase of laryngeal blood flow volume, whereas stimulation of the superior laryngeal nerve decreases 20% of the laryngeal blood flow. Findings of adrenergic activity just below the nasal membrane and intraepithelially in the laryngeal mucosa could be cautiously interpreted as a neuroepithelial body. Rogers and Haller (1978) found in the lung of Bafo Marinus neuroepithelial bodies. The characteristics of cells and their innervation were studied with the electron microscope and fluorescence microscopy. They proved that about 20% of neuroepithelial bodies are innervated solely by adrenergic nerve fibers.

They proposed that neuroepithelial bodies are receptors monitoring intrapulmonary P CO_2. It is logical to presume that many similar receptors should be found in the laryngeal and nasal mucosa.

CONCLUSIONS

1. The modified glyoxylic acid condensation fluorescent method is reliable for the demonstration of biogenic amines in human nasal and laryngeal mucosa.
2. The modified Karnovsky-Roots method could be used routinely for the clinical demonstration of cholinergic fibers in human nasal and laryngeal mucosa.
3. The significance and structure of neuroepithelial bodies in nasal and laryngeal mucosa should be further investigated.
4. Although functional studies could offer more precise information about respiratory mucosa innervation, further neurohistochemical studies on human material are invitable to standardize normal and pathological findings.

ZUSAMMENFASSUNG

Wir haben die histofluorescente Methode mit glyoxilitischer Säure nach Torre und Surgeon und die Karnovsky-Roots Methode für die Acethylcholinesterase Aktivität bei frischen Gewebe von Tieren und Menschen angewendet. Das Gewebe bei Menschen wurde während der operativen Eingriffen im HNO Gebiet entnommen und zwar von der nasalen, pharyngealen und hypopharyngealen Schleimhaut. Wir konnten bei Menschen, wie auch bei den Tieren adrenergische Axonen und Varicositäten im Bereiche der Gefässe beweisen, aber auch diffus in der nasalen, laryngealen und hypopharyngealen Mucosa gleich unter des Epithels oder auch intraepithelial. Wir fanden eine abundante cholinergische Innervation im Bereiche der Gefässe und der Drüsen, aber auch diffus zerstreut in den Geweben der nasalen, laryngealen und hypopharyngealen Schleimhaut.

REFERENCES

1. Änggård, A., 1974: The effect of parasympathetic nerve stimulation on the microcirculation and secretion in the nasal mucosa of the cat. Acta Otolaryng. 78, 98.
2. Änggård, A., Edwall, L., 1974: The effect of sympathetic nerve stimulation on the tracer disappearance rate and local blood content in the nasal mucosa of the cat. Acta Otolaryng. 77, 131.
3. Änggård, A., Densert, O., 1974: Adrenergic innervation of the nasal mucosa in cat. Acta Otolaryng. 78, 232.
4. Änggård, A., 1979: Vasomotor rhinitis – pathophysological aspects. Rhinol. 17, 31.
5. Karnovsky, M. J., Roots, L., 1964: A direct coloring thiocholine method for cholinesterases. J. Histochem. Cytochem. 12, 219.
6. Krajina, Z., Krmpotić, J., Večerina, S., Kostovic, I., Kelovic, Z., Risavi, R., Poljak, Z., 1979: Innervation of respiratory mucosa as revealed by come current histochemical techniques. World Congress of Allergology. Jerusalem.
7. Lundberg, J. M., Änggård, A., Fahrenkrug, J., Hokfelt, T., Muttg V., 1980: Vasoactive intestinal pclypeptide in cholinergic neurons of exocrine glands: Functional significance of coexiting transmitters for vasodilatation and secretion. Proc. Natl. Acade. Sci. USA. 77, 1651.
8. Nomura, Y., Matsuura, T., 1972: Distribution and clinical significance of the autonomic nervous system in the human nasal mucosa. Acta Otolaryng. 73, 493.

9. Rogers, D. C., Haller, J. C., 1978: Innervation and cytochemistry of the neuroepithelial bodies in the ciliated epithelium of the toad lung (Bufo Marinus). Cell Tiss. Res., 195, 395.
10. Shin, T., 1970: Vasomotor responses to laryngeal nerve stimulation. Arch. Otolaryng. 92, 257.
11. Torre, J. C., Surgeon, J. W., 1976: A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: the SPG method. Histochemistry. 49, 81.
12. Uddman, R., Malm, L., Sundler, F., 1981: Peptide containing nerves in the nasal mucosa. Rhinol. 19, 75.
13. Večerina, S., Krajina, Z., Zirdum, A., Poljak, Z., 1981: Pathophysiological mechanisms of some professional voice disorders: neurohistochemistry of normal laryngeal mucosa. Proceedings of the Congress of European Phoniatricians, Amsterdam.
14. Wilson, H., Yates. M. S., 1977: Sympathetic nerve pathways to the nasal vasculature of the cat. Acta Otolaryngol., 84, 278.

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