New data on the precise location of the lacrimo-muconasal nucleus of the rat

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Summary. Three methods (axonal degeneration, retrograde labelling with HRP and Golgi's silver impregnation) were used in the identification of a group of cells located in the ventrolateral part of the reticular formation of the pons, which are postulated to form the lacrimo-muconasal nucleus of the rat.

The parasympathetic neurons whose target organs are the lacrimal gland and the oronasal mucous membranes have often been described as part of the superior salivary nucleus[1,2]. On the other hand, the authors who consider it to be an independent nucleus do not agree on its location[3–5]. In this paper we present evidence for the precise localization of the nucleus lacrimo-muconasalis (n. lmn.) in the rat.

Materials and methods. 1. The infraorbital nerve was sectioned in 10 rats and the resulting degeneration in the brain stem, considered as indirect Wallerian degeneration, was studied by the Nauta, Fink-Heimer and Velayos-Lizarraga[6] methods. Mean survival time was 6 days.

2. In another group of 38 rats, the maxillary nerve was injected with 4 µl of a 50% saline solution of horseradish peroxidase (HRP), Sigma type VI. The injection site was close to the esphenopalatine ganglion, at the base of the orbit. After 37–62 h, the animals were perfused with a solution of 2.5% glutaraldehyde and 4% paraformaldehyde and processed by the technique of Llamas and Martínez Moreno[7].

3. The brain stems of 17 rats aged between 6 and 15 days were processed according to Valverde's modificaction of the rapid Golgi method, in order to study the morphology of neurons in the ventrolateral part of the pontine reticular formation and the initial course of their axons and processes. Abad Alegría's atlas of the brainstem of the rat was used to aid in the localization of the different nuclei[8].

Results. In the 1st group of animals, signs of degenerated fibres not attributable to a transganglionic degeneration, and therefore considered as indirect Wallerian degeneration, were observed in the ventrolateral part of the nucleus reticularis pontis caudalis between the most caudal part of the nucleus principalis or the cranial portion of the subnucleus oralis, and the nucleus facialis. It was sparse, extending over the dorsal part of the nucleus facialis, even between the facial motoneurons. After electrocoagulation of the Gasserian ganglion in previous experiments, we observed the presence of preterminal and terminal degeneration in the same area[9].

8 animals from the 2nd group showed HRP labelled neurons in this area. Topographically, the first neurons were observed between the ventromedial part of the nucleus principalis and the most caudal part of the superior olivary complex. The number of labelled cells increased caudally, reaching a maximum at the level of the cranial part of the subnucleus oralis, between its ventromedial border and the lateral part of the nucleus facialis, forming a compact group of about 50 cells. Some labelled cells were located between the facial motoneurons or behind the nucleus facialis. They decreased in size caudally, following the direction of the radix facialis (figure 1). The neurons were small or medium sized and elongated in shape (figure 2).

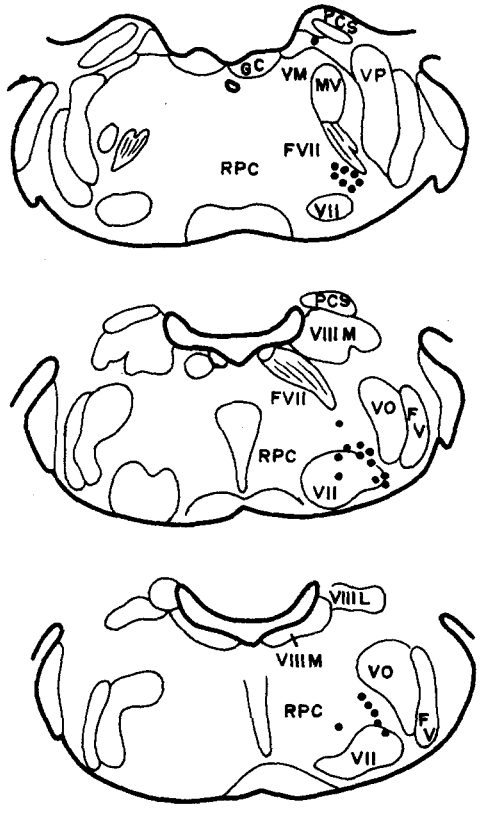

Fig. 1. Schema showing the distribution of the labelled neurons in the ventrolateral part of the pontine reticular formation (dots). Abbreviations: FV, tractus spinalis nervi trigemini; FVII, fibrae n. VII; GC, griseum centrale; MV, nuc. motorius nervi trigemini; PCS, pedunculus cerebellaris superior; VM, nuc. tractus mesencephalici nervi trigemini; VO, nuc. oralis tractus spinalis nervi trigemini; VP, nuc principalis nervi trigemini; VII, nuc facialis; VIII M, nuc. vestibularis medialis; VIII L, nuc. vestibularis lateralis; RPC, nuc reticularis pontis caudalis.

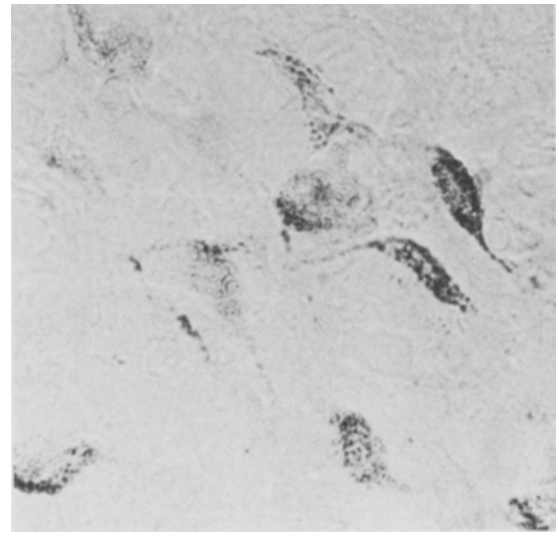

Fig. 2. HRP containing neurons in the ipsilateral pontine reticular formation (ventrolateral part).

It is noteworthy that this group of labelled neurons appeared when HRP was injected near the sphenopalatine ganglion, although in 1 case a neuron was observed at the same position after severance of fibres of the vidian nerve. The morphology of these neurons in the Golgi material was very similar to that revealed by the HRP group. Dendrites of these neurons were in relation to the cells of the subnucleus oralis and nucleus principalis; their axons went out with the facial root. No axons were observed entering the subnucleus oralis or nucleus principalis.

Discussion. The existence of a certain group of cells situated in the ventrolateral part of the reticular formation has been previously demonstrated[3-5]. This 'ventrolateral group' was considered to be part of the superior salivary nucleus[10], but in fact both salivary nuclei are located in the brainstem in a more dorsomedial position[2,3]. However, the precise location of the neurons innervating the oral and nasal mucosae had not yet been established. Our findings suggest that this ventrolateral group has this function. The presence of labelled neurons after HRP injection in the vicinity of the sphenopalatine ganglion strongly favours the hypothesis that the HRP was transported by the vidian, great petrosal and intermediate nerves as far as the brainstem. Moreover, the findings in the 1st group suggest that at least some of the fibres may run with the trigeminal nerve. Indirect Wallerian degeneration of vidian and intermediate nerve fibres would stop at the sphenopalatine ganglion, but that of trigeminal fibres would not. These degenerated fibres were present in the ventrolateral reticular formation. The close interrelationships between the ventrolateral group and the trigeminal nuclei were demonstrated by the Golgi material. This might explain the vasomotor phenomena which sometimes occur in trigeminal neuralgia. Moreover, physiological findings[11] suggest that this zone is involved in general autonomic efferent reactions. Histochemical studies show this zone to be extremely coincident with Dalström and Fuxe's A5[12,13], containing catecholamines and acetylcholine.

Taken together, these facts indicate that this ventrolateral group is an important individual parasympathetic center in the brainstem, functioning as an area of somatic and autonomic integration.

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The role of vitamin E in preventing the hemolysis of kid and chick erythrocytes with Tween 20

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Summary. Erythrocytes of vitamin E-deficient kids and chicks were hemolyzed at concentrations of Tween 20 above 1%. In vivo or in vitro uptake of vitamin E by the erythrocytes, or the addition of dithiothreitol or 2-mercaptoethanol, prevented the hemolysis with Tween 20.

Several hemolytic procedures[1-3] have been used in vitro to estimate the vitamin E status of rats. However, no such procedures have been reported for ruminants. In our preliminary work we tried to find a specific hemolytic agent for the erythrocytes of vitamin E-deficient kids, and among several detergents tested, Tween 20 seemed to be a promising one. In this paper we examined the hemolytic procedure with Tween 20 and confirmed its usefulness as a tool for detecting vitamin E deficiency in kids and chicks.

Materials and methods. 3-day-old Japanese meat-type kids were bottle-fed either a vitamin E-deficient or a vitamin E-supplemented diet reconstituted with warm water in the ratio of 1:5. 60 g of diet were given daily until the animals were 2 weeks old, then 75 g were given per day. The basic composition of these diets was: 87.2% dried skim milk, 10.0% lard, 2.0% lecithin, and 0.8% vitamin-mineral supplement[4] devoid of vitamin E. Only the vitamin E-supplemented diet contained 100 mg dl-α-tocopherol/kg. Rats and chicks were fed the same diets with the addition of 5% cellulose.

The standard hemolytic procedure used for the kids was as follows: 25 volumes of saline-phosphate buffer (pH 7.4)[2] containing 0.5 mM Na EDTA was added to 1 volume of heparinized blood and the mixture was centrifuged at 1000 × g for 10 min. The cell pellet was resuspended with the same volume of the above buffer and stored at 4 °C. A 0.5 ml aliquot of the cell suspension was mixed with 0.5 ml of 5% (v/v) Tween 20 in 0.9% saline and incubated at 37 °C for 15 min. Immediately after incubation, 2.5 ml of ice-cold saline-phosphate buffer was added to the incubation mixture and centrifuged. The optical density of the supernatant was measured at 540 nm. Percentage hemolysis was calculated by dividing the above optical density by the optical density of the completely hemolyzed sample and multiplying by 100. For the hemolytic tests of rat and chick

Means and standard deviations of percentage hemolysis with Tween 20 of erythrocytes of kids fed vitamin E-deficient or vitamin E-supplemented diets

Period of diet	Percentage hemolysis	
	Vitamin E-deficient diet	Vitamin E-supplemented diet
2 weeks	35±11 (4)	3±2 (3)
3 weeks	69± 7 (4)	7±5 (3)
4 weeks	79± 2 (4)	7±1 (3)
9 weeks	90± 4 (4)	5±3 (3)
14 weeks	89± 1 (3)	7±1 (2)

Figures in parentheses are number of animals used.