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Research Report

Determination of functionally essential neuronal population of the olfactory epithelium for nipple search and subsequent suckling behavior in newborn rats

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ABSTRACT

Neuronal population of the olfactory epithelium required for nipple search and subsequent suckling behavior was examined in newborn rats. After unilateral ablation of the olfactory bulb, different concentrations of $ZnSO_4$ were contralaterally injected into the nasal cavity to produce varying degrees of neuronal degeneration in the olfactory epithelium. The $ZnSO_4$-treatment resulted in two populations of pups. One exhibited suckling while the other did not, and intact olfactory receptor neurons were quantified immunohistochemically using an antibody for olfactory marker protein (OMP), a marker protein for olfactory receptor neurons. The total numbers of the OMP (+) cells in the $ZnSO_4$-treated pups with suckling capability ranged between 2457 and 4615, whereas those in the $ZnSO_4$-treated pups without suckling capability ranged between 112 and 2398. With the mean value (4969) of the total numbers of the OMP (+) cells of the normal/control pups assumed to represent 100%, the total numbers of the OMP (+) cells accounted for 49–93% in the suckling (+) group and 2–48% in the suckling (–) group. From these findings, we conclude that approximately 50% of neuronal population of the olfactory epithelium is a critical value to distinguish between the two groups with and without suckling capability in the unilateral olfactory system of newborn rats.

1. Introduction

Olfactory cues are essential for nipple search in newborn mammals (Bruno et al., 1980; Henning, 1980; Larson and Stein, 1984; Distel and Hudson, 1985; Risser and Slotnick, 1987a; Yokouchi et al., 2007), and thus removal of the bilateral olfactory bulb, a primary brain site of the olfactory system, results in suckling disturbance in rodent newborns (Distel and Hudson, 1985; Risser and Slotnick, 1987a; Yokouchi et al., 2007). However, the unilateral olfactory system is sufficient for suckling, since unilateral bulbectomy does not interfere with nipple attachment and leads to suckling behavior (Distel and Hudson, 1985; Risser and Slotnick, 1987a; Yokouchi et al., 2007).

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Abbreviations: OMP, olfactory marker protein; P, postnatal day

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Fig. 1 – Bulbar regions after ablation of the unilateral olfactory bulb at the rostral (A) and caudal (B) levels. The bulbectomized portion is occupied with amorphous tissue fragments (asterisks). Note the presence of the OMP (+) neural elements in the outermost olfactory nerve and glomerular layers of the unlesioned olfactory bulb. Scale bar = 100 μm.

Olfactory function is generally difficult to examine in neonatal animals, but suckling behavior appears to make it possible in consideration that newborns use olfactory cues for nipple attachment in rodents (Bruno et al., 1980; Distel and Hudson, 1985). The present study was aimed to determine neuronal population of the olfactory epithelium essential for suckling in newborn rats. For this purpose, we induced varying degrees of neuronal degeneration of the unilateral olfactory epithelium by various modes of intranasal injections of $ZnSO_4$ combined with unilateral olfactory bulbectomy contralateral to $ZnSO_4$ injections.

Since olfactory marker protein (OMP) is a well-established marker for olfactory receptor neurons (Farbman and Margolis, 1980; Monti Graziadei et al., 1980), we tried to reveal, by an immunohistochemical method using an anti-OMP antibody, the relationship between OMP (+) intact olfactory receptor neurons and olfactory function in terms of suckling capability. The minimum number of olfactory receptor neurons required for suckling will be determined in newborn rats.

2. Results

2.1. *Ablation of the unilateral olfactory bulb*

Fig. 1 shows bulbar regions of the $ZnSO_4$-treated pup with ablation of the unilateral olfactory bulb. The bulbectomized

Table 1 – Suckling behavior and OMP (+) cells of the experimental groups.

Normal and control* pups				$ZnSO_4$-treated pups									
No.	Suckling	Milk	OMP (+) cells	Treatment	No.	Suckling	Milk	OMP (+) cells	Treatment	No.	Suckling	Milk	OMP (+) cells
1	+	+	5303	2.5%×1	1	+	–	4009	5%×2	1	+	+	2457
2	+	+	5455		2	+	+	N.E.		2	+	–	4176
3	+	+	4825	2.5%×2	1	+	+	4615		3	+	+	3162
4	+	+	4330		2	+	+	3080		4	+	+	2740
5	+	–	N.E.		3	+	+	N.E.	5%×3	1	–	–	2217
6	+	+	N.E.	2.5%×3	1	+	–	4301		2	–	–	112
*7	+	+	4934		2	–	–	N.E.		3	–	–	150
*8	+	+	N.E.		3	–	–	N.E.		4	–	–	175
*9	+	–	N.E.		4	–	–	N.E.		5	–	–	191
					5	+	+	3738		6	–	–	2074
					6	–	–	2177		7	–	–	1043
					7	–	–	2398					
					8	–	–	N.E.					
					9	–	–	N.E.					