Conversion of types of proteins secreted by submandibular glands of rats during postnatal development

Kimio Abe*, Hiroshi Inoue**, Tetsuya Habu***,
Naoaki Sawamura*** and Satoru Naruse****

*Department of Oral Biochemistry (Chief: Prof. Y. Yokota), Gifu College of Dentistry, Gifu 501-02, **The First Department of Conservative Dentistry (Chief: Prof. H. Inoue),

The First Department of Prosthodontics (Chief: Prof. T. Habu) and *Department of Oral Pharmacology (Chief: Prof. S. Naruse), Fukuoka Dental College, 700, Ta, Sawara-ku, Fukuoka-shi 814-01, Japan

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Introduction

The submandibular glands of both young and adult rats secrete additional and different types of proteins in response to α -adrenoceptor agonists such as methoxamine, phenylephrine, noradrenaline, adrenaline, dopamine and α -methylnoradrenaline (α -mNA) at high doses as opposed to β - and β_2 -adrenoceptor agonists, namely, isoproterenol, procaterole and terbutaline and cholinergic agonists, carbachol, pilocarpine and methacholine¹⁻⁹). However, at relatively low doses the α -adrenoceptor agonists, except methoxamine at relatively low doses which can secrete the γ -types of proteins¹⁰), elicit secretion of proteins characteristic of β - and β_2 -adrenergic or cholinergic stimuli¹⁻⁹).

Conversion of the types of proteins from the α -types characteristic of α -adrenoceptor to the β -types characteristic of β -adrenergic and cholinergic receptors occurs in the adult submandibular glands of rats after injection of α -adrenoceptor antagonists, whether they preferentially inhibit α_1 - or α_2 -adrenoceptor, but not with muscarinic, nicotinic, dopaminergic, β_1 - and β_2 -adrenergic antagonists or adrenalectomy¹⁻¹⁰).

The present study, thus, was designed to determine whether conversion of the α -types to the β -types of proteins occurred or not in the submandibular glands of young rats

during postnatal development, using an α -adrenergic antagonist, phentolamine. In addition, we wished to determine the effects of phentolamine on flow rates and protein secretion by the submandibular glands of the young rats during postnatal development in combination with α -mNA.

Materials and Methods

Young male Sprague-Dawley rats 2 to 10 weeks of age were divided into experimental groups of about 5 to 7 animals. A 12 h light-dark cycle was maintained in the room by an automatic timing device with lights turned off at 6 p.m. and the animals had *ad libitum* access to MF rat chow (Oriental Kobokogyo, Tokyo) and water.

The rats were fasted from 9 a.m. to 5 p.m. prior to the collection of saliva, then anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg, i.p.), secured in a supine position with tape and tracheotomized to facilitate respiration during the saliva collection. Both submandibular ducts were cannulated introrally by the method of Yoshida *et al.*¹¹) With the exception of the initial two drops, which were discarded, submandibular saliva was collected for 1 h after i.p. drug administration. Saliva was collected into microcentrifuge tubes by the rats of 2 weeks of age and also into Pyrex-glass tubes by the rats of 3 to 10 weeks of age and kept on ice.

Volumes were estimated by weight, assuming the specific gravity of saliva to be 1.00.

As secretory stimulation, α -mNA (20 mg/kg), an α -adrenoceptor agonist to elicit secretion of saliva with the types of proteins characteristic of α -adrenoceptor stimulation as described previously⁶⁾, was used i.p. Phentolamine (25 mg/kg), an α -adrenoceptor antagonist, was also used in combination with α -mNA in any rat 2 to 10 weeks of age. After saliva collection, the sublingual glands were carefully dissected away from the submandibular glands and then the latter was removed and weighed.

Saliva samples were analyzed for total protein by the method of Lowry *et al.*¹²⁾ using casein as a standard and then, after addition of $\text{Na}_3\text{-EDTA}$ to avoid subsequent precipitation of calcium proteinate, were kept frozen at -20°C until lyophilization could be carried out. After reconstitution of the lyophilized saliva in water, disc electrophoresis was carried out in both anionic and cationic systems using 7.5 per cent polyacrylamide gels as described previously¹⁾. The gels were stained with Wool Fast Blue BL for the anionic gels and Fast Green for the cationic systems as described in detail previously¹⁾. The gels were, then, destained for 2 days with 1 per cent acetic acid for

anionic gels and 7 per cent acetic acid for the cationic gels.

Results and Discussion

For submandibular saliva elicited by i.p. injection of α -mNA in combination with phentolamine, the volumes, flow rates, the protein concentration and the amounts of protein secreted, whether they were expressed as mg/h or $\mu\text{g}/\text{mg}/\text{h}$, increased progressively with increasing weeks of age up to 6 or 7 weeks of age and then reached the plateau values in almost all parameters with a couple of exceptions at 8 weeks of age as shown in Table 1.

Salivary volumes, flow rates, the protein concentration and the amounts of protein secreted by the submandibular glands during the postnatal development were compared between those in α -mNA alone as reported recently⁷⁾ and those in α -mNA in combination with phentolamine as shown in Table 2. Phentolamine markedly but not completely inhibited salivary volumes by about 25 per cent of α -mNA-induced secretion from the submandibular glands in any week of age during the postnatal development, except at 2 weeks of age about 50 per cent inhibition was found. By phentolamine, salivary flow

Table 1 The effects of phentolamine in combination with α -methylnoradrenaline on salivary flow rate and protein secretion by the submandibular glands of rats during the postnatal development (means $\pm$ S.E.).

Age (weeks)	2	3	4	5	6	7	8	10
Volume of saliva (μl in 1 h)	39.3 $\pm$ 4.5	53.5 $\pm$ 9.1	106.7 $\pm$ 21.5	145.9 $\pm$ 8.0	270.6 $\pm$ 26.3	254.8 $\pm$ 20.5	226.4 $\pm$ 5.0	344.4 $\pm$ 25.9
Flow rate (nl/mg.min)	6.55 $\pm$ 0.74	6.88 $\pm$ 0.96	9.68 $\pm$ 2.06	11.40 $\pm$ 0.47	15.73 $\pm$ 1.42	13.46 $\pm$ 1.17	12.52 $\pm$ 0.60	14.85 $\pm$ 1.15
Protein conc. (g/100 cm ³)	1.5 $\pm$ 0.2	2.8 $\pm$ 0.1	3.2 $\pm$ 0.2	3.7 $\pm$ 0.2	4.7 $\pm$ 0.1	6.3 $\pm$ 0.1	4.2 $\pm$ 0.2	5.2 $\pm$ 0.1
Protein secreted (mg/h)	0.59 $\pm$ 0.08	1.53 $\pm$ 0.27	3.44 $\pm$ 0.76	5.39 $\pm$ 0.94	12.63 $\pm$ 1.07	16.08 $\pm$ 1.50	9.60 $\pm$ 1.49	17.85 $\pm$ 1.22
($\mu\text{g}/\text{mg}/\text{h}$)	5.80 $\pm$ 0.68	11.80 $\pm$ 1.75	18.78 $\pm$ 4.38	25.30 $\pm$ 2.50	44.05 $\pm$ 3.43	50.92 $\pm$ 5.00	31.54 $\pm$ 0.85	44.25 $\pm$ 4.57
No. of rats	6	7	5	5	5	6	5	5

Table 2 Comparison of flow rate and protein secretion by the submandibular glands of rats in response to α -methylnoradrenaline with and without phentolamine during the postnatal development.

Age (weeks)	2	3	4	5	6	7	8	10
Volume of saliva (μ l in 1 h)	50.3	28.8	25.1	25.3	26.2	27.2	24.9	29.7
Flow rate (nl/mg.min)	47.6	36.2	30.4	32.0	34.9	36.6	43.7	43.4
Protein conc. (g/100 cm ³)	223.3	582.5	541.4	436.9	503.1	596.1	349.9	357.3
Protein secreted (mg/h)	122.9	170.0	133.3	119.2	151.3	173.1	105.4	130.9
(μ g/mg.h)	114.2	211.8	123.8	148.2	201.0	224.2	175.1	178.9
No. of rats Without*	13	11	16	17	11	12	11	13
With**	6	7	5	5	5	6	5	5

The experimental values are all expressed as a percentage of the controls (without)

*; denotes α -methylnoradrenaline stimulation alone

**; denotes α -methylnoradrenaline stimulation in combination with phentolamine

Fig. 1 Anionic disc gel electrophoresis on 7.5 per cent polyacrylamide gels of submandibular saliva of rats 2 to 10 weeks of age during the postnatal development in response to α -mNA in combination with phentolamine. The positive pole is at the bottom.

rates were also greatly, but not completely, inhibited in almost any week of age. Inhibition rates of salivary volumes secreted by the submandibular glands, thus, appeared to be

independent on any week of age except 2 weeks of age during the postnatal development when phentolamine was used. A similar finding was made previously with α -mNA in

2 3 4 5 6 7 8-10(W)
 α -mNA + phentolamine, Submandibular, Cationic gel (7.5%)
 Fig. 2 Cationic disc gel electrophoresis on 7.5 per cent polyacrylamide gels of submandibular saliva of rats 2 to 10 weeks of age during the postnatal development in response to α -mNA in combination with phentolamine. The negative pole is at the bottom.

combination with phentolamine in adulthood as described previously⁶⁾.

In contrast, phentolamine markedly elevated the protein concentration by about 5 to 6 times higher than that elicited by α -mNA alone 3 to 7 weeks of age although at 2, 8 and 10 weeks of age the elevation of the protein concentration was found to be a little bit less than that of the former weeks of age during the postnatal development. The amounts of protein secreted, whether they were expressed as mg/h or μ g/mg/h, were also elevated by phentolamine in any week of age during the postnatal development. Similar findings were made previously⁶⁾ with α -mNA in combination with phentolamine, phenoxybenzamine, dihydroergotamine, tolazoline and yohimbine, which could preferentially block α_1 - or α_2 -adrenoceptors, and with atropine in contrast with propranolol in the adult rats.

Conversion of the α -types to the β -types of proteins in submandibular saliva of rats in any week of age during the postnatal development completely occurred electrophoretically in both anionic (Fig. 1) and cationic (Fig. 2) systems using 7.5 per cent polyacrylamide gels when phentolamine was used in combination with α -mNA although at 2 weeks of age the types of proteins elicited by α -mNA and isoproterenol were not greatly different as shown previously⁷⁾. A similar finding was made with α -mNA in combination with phentolamine of saliva by the adult rats⁶⁾.

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