[Chem. Pharm. Bull. 30(3)1059—1062(1982)]

Studies on LM Protein appearing in Submandibular Glands of Isoproterenol-treated Rats. III. Some Aspects of Its Formation

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(Received August 5, 1981)

A single stimulation with isoproterenol (IPR) resulted in a 1.5-fold increase in the total protein in saliva from rat submandibular glands at 1 day after the injection. Large mobile (LM) protein began to be secreted from the glands early after IPR injection, reaching a maximum at 2 days after injection, when the total protein in saliva had returned to the normal level. The secretion of this protein continued for 22 days after injection, indicating that the life of LM protein template is long or that the effect of IPR remains during this period.

The increase of LM protein after IPR stimulation was markedly suppressed by β-adrenergic receptor blocking drugs. When both an α-adrenergic receptor blocking drug and adrenaline were injected together into rats, a small amount of the LM protein appeared and an increase in the wet weight of submandibular glands was seen. Repeated injection of these drugs resulted in a remarkable increase of LM protein. These results suggest that the appearance of LM protein was not a consequence of direct action of IPR but was due to a stimulatory effect of IPR on β-adrenergic receptors and that chronic blocking of α-adrenergic receptors has a significant stimulative effect on the production of LM protein through β-adrenergic receptors.

Keywords——submandibular gland; isoproterenol; LM protein; saliva protein; β-adrenergic receptor

Introduction

Menaker *et al.*[1] reported that a protein which was not observed in normal rats appeared in the saliva of rats administered isoproterenol (IPR) twice daily, and this protein was named large mobile (LM) protein. A different protein was characterized by Fernandez-Soresen and Carlson[2] in the parotid glands of rats treated similarly. However, these proteins have not been fully characterized.

In the previous paper, we reported the purification and characterization of the LM protein isolated from IPR-treated rat submandibular saliva.[3,4] However, the mechanism of its formation has not been fully clarified. This paper describes some pharmacological aspects of the appearance of LM protein on IPR stimulation.

Materials and Methods

Male Sprague-Dawley rats (250—300 g weight) kept as described previously[3] were injected intraperitoneally (*i.p.*) with *dl*-isoproterenol–HCl (IPR) (Nakarai Chemicals, Kyoto) at 6 p.m. and saliva was collected at 3, 5, 7, 9, 12, 18 and 24 h, and then at 2, 3, 6, 9, 12, 22 and 23 d after IPR injection. Control rats received *i.p.* an equal volume of sterilized saline. When the effect of pre-administration of β-adrenergic receptor blocking drugs was to be examined, the rats were divided into four groups. One group was injected *i.p.* with 20 mg/kg of IPR at 6 p.m. Two other groups were injected *i.p.* with either 27 mg/kg of propranolol–HCl (Sigma Chemical) or 46 mg/kg of dichloroisoproterenol–HCl (DCI) (Sigma Chemical) 30 min before IPR injection. The control group was injected with sterilized saline. Additional experiments were performed; one group was injected *i.p.* with 2 mg/kg of adrenaline–HCl (Katayama Chemical, Osaka) and two other groups were injected *i.p.* with 10 mg/kg of phenoxybenzamine (Tokyo Kasei, Tokyo) 30 min before adrenaline injection once a day for 1 or 4 d. Rat submandibular saliva was collected as described previously.[3] After the collection of saliva, the right and left submandibular glands were dissected and the wet weights were recorded.

Protein was measured according to the method of Lowry *et al.*[5] Bovine serum albumin (Sigma Chemical) was used as a standard protein. Polyacrylamide gel electrophoresis was performed by a modification of the method of Davis,[6] as described previously.[3] The gels were stained according to the method of Mayer and Lamberts.[7] The concentration of LM protein in submandibular saliva was determined by the single radial immunodiffusion method[8] as described previously.[3]

Results

The time courses of the secretion of total saliva protein and LM protein from rat submandibular glands following a single IPR injection are shown in Fig. 1. The total saliva protein had decreased remarkably at 3 h after IPR injection, but it increased to a level 50% above the control at 1 d and then it returned to the control level at 2 d after injection. The composition of saliva proteins was analyzed by polyacrylamide gel electrophoresis (data not shown). The LM protein band appeared in the saliva collected 9 h after IPR injection and its intensity increased as time passed. The amount of LM protein was measured by a single radial immunodiffusion method[3] (Fig. 1). Although the total saliva protein reached its maximum at 1 d after IPR injection, the LM protein reached its maximum at 2 d after injection. Then, the amount of LM protein decreased slowly during 22 d and it finally disappeared at 23 d after injection. Thus, the LM protein behaved differently from the total saliva protein. Except for the appearance of the LM protein, no qualitative difference was observed between the electrophoretic patterns of the IPR-administered group and those of the control group (data not shown).

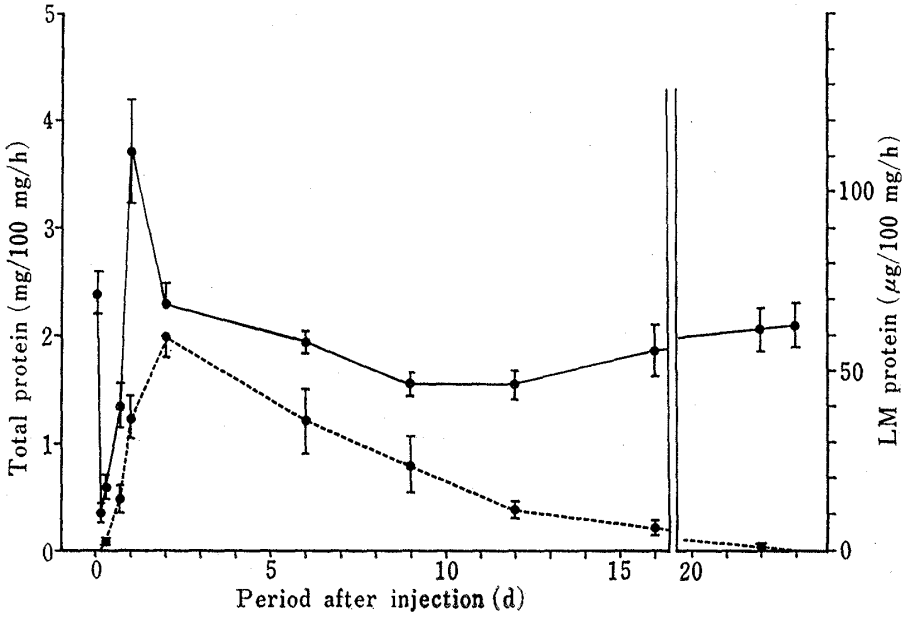

Fig. 1. Time Course of Protein Secretion from the Rat Submandibular Glands following IPR Injection

Saliva was collected from the rats after IPR stimulation (20 mg/kg) for 90 min at various times after IPR injection, and the amounts of total saliva protein (mg)/glands (100 mg)/h (●—●) and LM protein (μg)/glands (100 mg)/h (●--●) were determined as described in the text. At least five animals were used to obtain each point (which represents the mean±S.E.).

As shown in Table I, the wet weight of submandibular glands and the amount of total protein increased after a single injection of IPR. When either DCI or propranolol was injected 30 min before IPR injection, the increase of the wet weight of submandibular gland was not suppressed, but the increase of the total saliva protein was inhibited slightly. On the other hand, these β-adrenergic receptor blocking drugs inhibited the increase of LM protein by more than 90%. A single injection of adrenaline or adrenaline plus phenoxybenzamine

Table I. Effects of Various Drugs on the Secretion of Proteins from Rat Submandibular Glands

Drug	No. of rats	Protein concentration (%) Mean ± S.E.	Total protein / Submandibular gland (mg/100 mg/h) Mean ± S.E.	LM Protein / Submandibular gland (μg/100 mg/h) Mean ± S.E.	Submandibular gland / Body weight (mg/100 g) Mean ± S.E.
Saline	9	3.81±0.24	2.44±0.06	~0	125±6
IPR	8	4.10±0.43	3.72±0.29	37.5±3.9	142±5
DCI+IPR	8	4.14±0.24	3.27±0.07	2.0±0.4	138±7
Propranolol+IPR	7	3.51±0.16	2.98±0.22	2.4±0.3	142±4
Adrenaline	7	3.76±0.34	2.40±0.10	~0	124±4
Phenoxybenzamine+adrenaline	7	3.75±0.24	2.49±0.09	2.0±0.4	129±5
Phenoxybenzamine+adrenaline[a)]	7	4.07±0.42	3.85±0.30	323.0±80	158±6

The groups of rats were injected with various drugs. Dichloroisoproterenol-HCl (DCI)(46 mg/kg) or propranolol-HCl (27 mg/kg) was injected 30 min before IPR injection. Phenoxybenzamine (10 mg/kg) was injected 30 min before adrenaline-HCl (2 mg/kg).
a) Phenoxybenzamine and adrenaline were administered together for four consecutive d. Saliva was collected for 90 min at 1 d after the last injection from each group of rats given IPR (20 mg/kg).

did not affect the wet weight of submandibular gland or the total saliva protein significantly, although the LM protein was detectable in the latter case. When phenoxybenzamine and adrenaline were used in combination for four consecutive d, the wet weight of submandibular glands and the total saliva protein increased significantly, and the amount of the LM protein increased drastically.

Discussion

The total protein in submandibular saliva of rats chronically treated with IPR for 1 week increased 2- to 3-fold.[3,9)] A single injection of IPR increased the amount of total submandibular saliva protein 1.5-fold (Fig. 1). These results suggested that IPR, a β-adrenergic receptor agonist, not only accelerates the secretion of submandibular protein but also stimulates the synthesis of externally secreted protein. The fact that a single injection of IPR has a prolonged effect on LM protein indicates that the life of protein template is long and/or that the effect of IPR stimulation remains for a long time.

DCI, a β-adrenergic receptor blocking drug, is known to inhibit the increase of DNA synthesis[10)] and the increase in the weight of rat submandibular gland produced by chronic IPR administration.[11)] As shown in Table I, the pre-administration of both DCI and propranolol inhibited the increase of the total saliva protein, but the increase in the wet weight of the gland was not suppressed. The appearance of the LM protein after IPR stimulation was also inhibited by those β-adrenergic receptor blocking drugs. These results indicate that the increase in the total saliva protein and the appearance of the LM protein induced by IPR occur through β-adrenergic receptors.

On the other hand, Sellinger and Naim[12)] have reported that the DNA synthesis in the rat parotid gland is not stimulated by adrenaline. The LM protein did not appear after adrenaline stimulation. However, the LM protein appeared when adrenaline was injected 30 min after the injection of phenoxybenzamine, an α-adrenergic receptor blocking drug. Furthermore, the wet weight, the total saliva protein and the LM protein in the rat submandibular glands increased remarkably on repeated administration of adrenaline plus phenoxybenzamine. Thus, blocking of α-adrenergic receptors has a significant stimulative effect on the production of some submandibular proteins stimulated through β-adrenergic receptors.

Acknowledgement I wish to thank Prof. Harumi Okuyama, the Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, for his help in preparing the manuscript.

References and Notes

1) L. Menaker, J.H. Sheetz, C.M. Cobb, and J.M. Navia, *Lab. Invest.*, **30**, 341 (1974).
2) A. Fernandez-Soresen and D.M. Carlson, *Biochem. Biophys. Res. Commun.*, **60**, 249 (1974).
3) Y. Naito, *Chem. Pharm. Bull.*, **29**, 1365 (1981).
4) Y. Naito and I. Suzuki, *Chem. Pharm. Bull.*, **29**, 1373 (1981).
5) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
6) B.J. Davis, *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).
7) T.S. Meyer and B.L. Lamberts, *Archs Oral Biol.*, **13**, 839 (1968).
8) G. Mancini, A.O. Carbonara, and J.F. Heremans, *Immunochemistry*, **2**, 235 (1965).
9) K. Abe, R. Fujita, Y. Yokota, and C. Dawes, *Jap. J. Oral Biol.*, **20**, 521 (1978).
10) T. Barka, *Exp. Cell Res.*, **37**, 662 (1965).
11) P. Pohto, *Acta Odont. Scand.*, **24**, Suppl., 45 (1966).
12) Z. Selinger and E. Naim, *Biochim. Biophys. Acta*, **203**, 335 (1970).