Conversion of the types of salivary proteins from the α - to β -type by the submandibular glands of adult rats

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Introduction

Rat submandibular glands can usefully be utilized to differentiate the effects of various exogenously-applied catecholamines¹⁻⁴. The glands of young and adult rats secrete additional and different types (the α -type) of proteins in response to α_1 -adrenoceptor agonists, namely, methoxamine except in low doses, phenylephrine, noradrenaline, adrenaline, dopamine and α -methylnoradrenaline at optimal doses¹⁻⁵. In contrast, β_1 - and β_2 -adrenoceptor agonists such as isoproterenol, procaterole and terbutaline, cholinergic stimulants, carbachol, pilocarpine and methacholine and also α_1 -adrenoceptor agonists in low doses, except that methoxamine secretes another type (the γ -type) of proteins at relatively low doses, can secrete the β -type of proteins by the submandibular glands of young and adult rats¹⁻⁵.

Conversion of the types of proteins from the α - to β -type occurs in both young and adult submandibular glands of rats after injection of α -adrenoceptor antagonists, whether they preferentially block α_1 - or α_2 -adrenoceptors. These findings have clearly been elucidated in detail in the results in response to methoxamine⁶, dopamine³ and α -methylnoradrenaline^{4,5}. In contrast, any conversion of the types of proteins from the α - to β -type does not occur in the submandibular glands of rats in response to those α_1 -agonists in combination with muscarinic,

nicotinic, dopaminergic, β_1 - or β_2 -adrenergic blocking agents^{1,3,4,6,7} and adrenalectomy². Little information, however, is available with regard to those with noradrenaline.

The present study thus was designed to elucidate whether conversion of the α - to β -type of proteins occurred in the submandibular glands of adult rats in response to noradrenaline (2 mg/kg) in combination with various types of blocking agents. In addition, we wished to determine in detail, by use of various types of antagonists, the relative importance of the different types of receptors for the secretory effects of noradrenaline *in vivo* experiment and also of methoxamine with different nucleotides *in vitro* experiment.

Materials and Methods

Saliva collection—Adult male and female Sprague-Dawley rats of weight 200-550 g were distributed into experimental groups of about 5 to 8 animals. A 12 hr light-dark cycle was maintained by an automatic timing device with lights turned off at 1800 hr and the animals had access, *ad libitum*, to Oriental MF rat chow (Oriental Kobokogyo, Tokyo) and water.

The rats were fasted from 0900 to 1700 hr prior to the collection of saliva, then anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg body weight, i.p.), secured in the supine position with tape and tracheotomized to facilitate respiration during the saliva col-

lection. Both submandibular ducts were cannulated intra-orally by the method of Yoshida *et al.*⁸⁾. With the exception of the initial two drops, which were discarded, submandibular salivas were collected for 1 hr after i.p. drug administration. Saliva was collected into pyrex glass tubes kept in ice and volumes were estimated by weight, assuming the specific gravity of saliva to be 1.00. Then, noradrenaline was dissolved in isotonic saline and employed at doses of 2 mg/kg, i.p. Various blocking agents were used including cholinergic antagonists (atropine sulfate, 1 mg/kg and hexamethonium, 50 mg/kg), α_1 - and α_2 -adrenoceptor blockers (phentolamine, 50 mg/kg; phenoxybenzamine, 50 mg/kg; dihydroergotamine, 50 mg/kg; tolazoline, 50 mg/kg; yohimbine, 10 mg/kg and dibenamine, 100 mg/kg), β_1 -, β_2 - and non-selective β -adrenergic antagonists (ICI 118551⁹⁾, 10 mg/kg; metoprolol¹⁰⁾, 10 mg/kg and alprenolol, 10 mg/kg) and i.v. haloperidol (2 mg/kg) as a dopaminergic antagonist³⁾. In addition, gamma-aminobutyric acid (GABA), suggested as a possible inhibitory transmitter for the synaptic transmission at various sites, was used in doses of 50 mg/kg, i.p. All these blocking agents, except haloperidol, were injected i.p. 30 min prior to i.p. administration of noradrenaline (2 mg/kg). After saliva collection, the sublingual glands were carefully dissected away from the submandibular glands and then the latter was removed and weighed.

In vitro experiments using slices of the submandibular glands of rats, methoxamine (50 μ M) was used in combination with different nucleotides (ATP, ADP, AMP, dbcAMP; GTP, GDP, GMP, dbcGMP; CTP, CDP, CMP; UTP, UDP, UMP and IDP). These nucleotides were used in doses of 50, 100 and 150 μ M. Slices were made using Tissue chopper (McIlwain, England). Both glands from 3 rats were prepared for each experiment. The glands after removal from the rats were kept at 37°C in Krebs-Ringer bicarbonate glucose (5.7 mM) medium under an atmosphere of 95% O₂ and 5% CO₂ throughout all stages of the experiment. Slices were preincubated separately in nitrocellulose test tubes containing 5 ml

of the medium and taken for the experiment after 15 min. After slices were washed 3 times with fresh medium, slices from one tube were collected by decantation of the medium and transferred to another tube containing 5 ml of fresh medium. Protein release was induced by adding methoxamine (50 μ M) in combination with each nucleotide with different doses into fresh medium. After 15 min incubation, the solution was collected, dialyzed against distilled water and lyophilized. All experiments were followed by the method as described by Batzri *et al.*¹¹⁾. *Saliva analyses*—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 Na₃-EDTA to avoid subsequent precipitation of calcium proteinate (Dawes)¹³⁾, were kept frozen at -20°C until lyophilization could be carried out. After reconstitution of the lyophilized samples *in vivo* and *in vitro* experiments, disc (7.5 and 15% anionic systems) and SDS (10%)—polyacrylamide gel electrophoreses were carried out as described previously^{6,14)}. Amino acid composition of some saliva samples was analyzed by an amino acid analyzer (JLC-6AH, Nihondenshi, Tokyo) as described in detail by Abe *et al.*¹⁵⁾. Assessed by these criteria, secreted proteins were classified as the α - or β -type.

Results and Discussion

Effects of noradrenaline on the secretory response in combination with various types of blocking agents—The secretory effects of noradrenaline in combination with various α_1 - or α_2 -adrenoceptor antagonists, cholinergic blockers and haloperidol are shown in Table 1. The volumes and flow rates were significantly reduced with all of α -adrenoceptor antagonists except dibenamine and haloperidol whereas the protein concentration increased significantly with phentolamine, dibenamine and haloperidol. The total amounts of protein secreted, whether they were expressed as mg/hr or μ g/mg/hr, were significantly reduced with α_1 - or α_2 -adrenoceptor antagonists except phentolamine and dibenamine. In contrast, dibenamine sig-

Table 1 The effects of noradrenaline (2 mg/kg) in combination with α -adrenergic, cholinergic and dopaminergic antagonists on salivary flow rate and protein secretion by the submandibular glands of male adult rats

	I	II	III	IV	V	VI	VII	VIII	IX
Dose (mg/kg)	50	50	50	50	10	100	1	50	2, i. v.
Volume of saliva (μ l in 1 hr)	29.0††	35.2††	22.8††	45.1**	19.4††	132.7	101.9	108.5	46.3**
Flow rate (nl/mg/min)	31.9††	32.9††	19.6††	50.0	17.2††	125.2	99.2	106.8	43.5*
Protein conc. (g/100 cm ³)	188.9**	122.2	140.7	125.9	103.7	177.8*	129.6	77.8	211.1**
Protein secreted (mg/hr)	55.6	43.6*	31.8††	59.1	19.9††	233.0††	136.2	88.5	103.1
(μ g/mg/hr)	59.6	39.6*	26.4††	61.8	17.3††	227.1*	129.1	83.9	97.1
Types of proteins	β	β	β	β	β	α	α	α	α
No. of rats	5	6	5	5	5	8	6	5	5

The experimental values are all expressed as the percentage of the controls.

*=P<0.05; **=P<0.02; ††=P<0.001 by unpaired t-test.

I=Phentolamine; II=Phenoxybenzamine; III=Dihydroergotamine; IV=Tolazoline; V=Yohimbine; VI=Dibenzamine; VII=Atropine; VIII=Hexamethonium; IX=Haloperidol.

Table 2 The effects of noradrenaline (2 mg/kg) in combination with β -adrenergic antagonists and GABA on salivary flow rate and protein secretion by the submandibular glands of female adult rats

	ICI 118551	Metoprolol	Alprenolol	GABA*
Dose (mg/kg)	10	10	10	50
Volume of saliva (μ l in 1 hr)	48.4††	12.9††	2.6††	78.4
Flow rate (nl/mg/min)	53.7††	10.9††	2.4††	71.3
Protein conc. (g/100 cm ³)	25.0††	62.5	100.0	93.8
Protein secreted (mg/hr)	13.3††	6.0††	2.9††	68.7**
(μ g/mg/hr)	45.1††	4.8††	7.5††	59.3**
Types of proteins	α	α	α	α
No. of rats	7	3	3	5

The experimental values are all expressed as the percentage of the controls.

*=gamma-aminobutyric acid; **=P<0.02; ††=P<0.001 by unpaired t-test.

nificantly increased them.

The secretory effects of noradrenaline in combination with β_1 -, β_2 - or non-selective β -adrenoceptor antagonists and GABA are shown in Table 2. The volumes, flow rates and the total amounts of protein secreted were significantly reduced with β_1 -, β_2 - or non-selective β -adrenoceptor antagonists. In contrast, the protein concentration was not significantly reduced with any antagonists

except that with ICI 118551, as a selective β_2 -antagonist, it was significantly reduced. The total amounts of protein secreted were significantly reduced with GABA by the submandibular glands of rats in response to noradrenaline whereas the volumes, flow rates and the protein concentration were not significantly reduced with GABA. From these results, the exogenously-applied noradrenaline could be assumed to correlate with

the α_1 -, α_2 -, β_1 - or β_2 -adrenoceptors as well as the dopaminergic receptor, respectively. However, it is very important to consider that observed effects on the salivary gland do not necessarily reflect the direct action on the gland of noradrenaline injected i.p. Once injected, noradrenaline could theoretically act on endocrine glands, autonomic ganglia or neurons, as confirmed presently with GABA, to release endogenous hormones or transmitters which could then modify salivary gland physiology.

Conversion of the types of proteins from the α - to β -type—In both the 7.5 and 15% anionic and 10% SDS-polyacrylamide gel systems, electrophoretic patterns characteristic of α -proteins^{1,2)} were produced using submandibular saliva elicited by noradrenaline at doses of 2 mg/kg with and without cholinergic blockers, haloperidol and any type of β -adrenoceptor antagonists as shown in Tables 1 and 2. These findings were quite similar to those described previously^{2-4,6)}. Conversion of the α - to β -type of proteins was observed in salivas elicited by noradrenaline (2 mg/kg) in combination with any of α -adrenoceptor antagonists except dibenamine as shown in Tables 1 and 2. These findings were consistent with our previous results²⁻⁶⁾ except dopamine in combination with dibenamine³⁾. Electrophoretograms of α - and β -type proteins have previously been published^{1,14)} as have actual photographs of the stained gels as for disc electrophoreses^{2,6)} and also as for SDS-polyacrylamide gels⁶⁾. Amino acid composition of α - and β -type proteins has previously been published and compared^{4,16)}.

In vitro experiments, none of the nucleotides, at least tested here, converted the α - to β -type of proteins released from slices of the submandibular glands of rats. These findings were quite similar as those described previously⁶⁾ using any kind of secretagogues with and without an α -adrenergic antagonist, phentolamine. It is very surprising to note that, *in vitro* experiments, proteins induced by slices of the submandibular glands of rats were mainly characteristic of α -proteins on SDS-polyacrylamide gels and any conversion from the α - to β -type of

proteins was not observed at all. Further studies are, therefore, necessary to elucidate the secretory mechanisms of α -proteins by the submandibular glands of rats *in vitro* experiments.

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