Effects of Dobutamine and Terbutaline on the Secretion of Glycoproteins from the Acinar Cells of the Rat Submandibular Gland

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Abstract—The actions of dobutamine (DOB) and terbutaline (TER) on the secretion of marker glycoprotein (GP) from the secretory cells of the glands and secretion of fluid from rat submandibular gland (SMG) were investigated in combination with two antagonists, metoprolol (MET) and ICI-118551 (ICI). The ED₅₀ value of fluid secretion was 8.7 mg/kg for DOB and 5.9 mg/kg for TER. MET, administered prior to either agonists at a dose of 40 mg/kg, inhibited the fluid secretion. However, the blocking effects of ICI were considerably lower than those of MET. The electrophoretic profiles of GP in DOB-evoked saliva were similar to those in TER-evoked saliva, and included two characteristic main bands of GP I (130 KDa) and GP IV (21.5 KDa) from the acinar cells and a minor band of GP III (31 KDa) which originated in the cells of the granular tubules. When MET was administered at a dose of 5 mg/kg prior to DOB, the intensity of band I decreased, whereas that of band III did not change. These results showed that the SMG of rats contains both β_1 - and β_2 -adrenoceptors and that β -receptors which mediate the secretion of GP from the acinus and fluid from the gland are mainly of the β_1 -subtype.

It is generally recognized that β -adrenoceptors can be divided into two subtypes: the β_1 -subtype, which mediates cardiac stimulation and lipolysis, and the β_2 -subtype, which mediates the relaxation of bronchial and vascular smooth muscle. These subtypes were identified by experiments using a series of sympathomimetic amines (1, 2). This classification is supported by results of experiments with agonists of both β_1 - and β_2 -receptors and with their antagonists which have recently been developed. In the rat submandibular gland, the β -adrenoceptors which mediate secretion of fluid (3–6) and protein (3) and glandular enlargement (7–9) are mainly of the β_1 -subtype, which mediates secretion of amylase from the rat parotid gland (10–13).

We reported previously that the species of secreted glycoproteins differ markedly between the acini and granular convoluted tubules of the rat submandibular gland (14). We found that characteristic glycoproteins in

the acini are secreted into the saliva after stimulation by the non-specific β -adrenergic agent isoproterenol (15), as well as after stimulation by the cholinergic agent pilocarpine (16) and by substance P (17), whereas glycoproteins characteristic of the granular convoluted tubules are secreted in response to the α_1 -adrenergic agent, methoxamine (18, 19).

We are interested in assaying the specificity of the action of β_1 and β_2 -adrenergic agonists on secretory cells by analysis of the marker glycoproteins contained in the acinus and granular convoluted tubules of the rat submandibular gland. In the present study, we have examined the specificity of the actions of dobutamine, a β_1 -agonist, and terbutaline, a β_2 -agonist, on the secretion of marker glycoproteins, protein and fluid from the submandibular glands of rats, using metoprolol, a selective β_1 -antagonist, and ICI-118551, a selective β_2 -antagonist.

Materials and Methods

Collection of submandibular saliva: Male Sprague-Dawley rats, eight weeks of age, were fasted but given water ad libitum for the 24 hours prior to examination. Each rat was anesthetized with sodium pentobarbital (50 mg/kg b. wt., i.p.) and placed on a heating pad maintained at 37°C. The excretory ducts of the sublingual gland were ligated after being separated from the adherent tissues around the submandibular gland. The trachea was cannulated with a polyethylene tube (MRC, 2×2.7 mm). 1) Either dobutamine or terbutaline was administered intraperitoneally in doses which ranged from 5 to 40 mg/kg b. wt., and submandibular saliva was collected from the tip of the ductal cannula with a capillary micropipette (Drummond Microcaps, 10 and 20 μ l) for periods of 5 min over the course of 30 min. 2) Metoprolol or ICI-118551 at doses of 0.2, 1.0 and 5.0 mg/kg b. wt. was injected intravenously 30 min prior to injection of each agonist at a dose of 40 mg/kg b. wt. After the injection of each of the agonists, submandibular saliva was collected as described above. At the end of each experiment, the submandibular glands were carefully removed, and flow rates were then calculated as the volume of fluid elicited per minute per milligram of wet weight of each gland.

Preparation of functional segments: Parenchymal components from the rat submandibular gland were isolated by the method of Masuhara and Iwabuchi (14). Rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and the submandibular gland was perfused via the carotid artery with a solution of collagenase which consisted of 0.1% collagenase (Sigma, Type II), 1.0 mM CaCl_2 and 0.1% bovine serum albumin in modified Hanks' medium (137 mM NaCl, 5 mM KCl, 0.8 mM MgSO_4 , 0.33 mM NaH_2PO_4 , 0.44 mM KH_2PO_4 , 1 mM MgCl_2 and 10 mM Tris-HCl, pH 7.4). The submandibular gland was immediately removed, sliced, and then incubated for 120 min at 37°C in an aliquot of the same collagenase solution in an atmosphere of 95% O_2 plus 5% CO_2 . The slices were rinsed with ice-cold modified Hanks' solution to remove col-

lagenase. Each segment of the acini and granular convoluted tubules was dissected out with needles under a stereomicroscope, and each dissociated segment was collected in a siliconized capillary tube (Drummond Microcaps, 50 μ l).

SDS-polyacrylamide micro-disc electrophoresis: Each segment was dissolved in an equal volume of 6% (w/v) sodium dodecyl sulphate (SDS) solution which contained 10% 2-mercaptoethanol and then heated at 90°C for 3 min. The protein content of each of the samples was determined by the method of Lowry et al. (20) with bovine serum albumin as the standard. One μ l of each sample, containing 1% (w/v) SDS, 5% 2-mercaptoethanol and 20% glycerol, was applied to the top of a 4–40% continuous gradient polyacrylamide disc gel, in a 10 μ l capillary tube, as described by Rüchel et al. (21). Electrophoresis was performed at room temperature at 60 V for 60 min in 50 mM Tris-glycine buffer (pH 8.4) in 0.1% SDS. The apparent molecular weights of the glycoproteins detected on the densitometric scan were estimated from the relative positions of myosin, β -galactosidase, phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α -lactalbumin, which were used as molecular weight markers. Gels were stained either with 0.2% Coomassie Brilliant Blue R-250 for protein or with periodic-Schiff's reagent (PAS) for glycoprotein, and then destained in 7% acetic acid. The patterns of glycoproteins and proteins on the gels were recorded photographically and by spectrophotometric traces of the gels made with a Joyce-Loebl 3CS microdensitometer at a wave length of 595 nm, for gels stained with Coomassie Blue, and at 550 nm, for those stained with PAS.

Drugs: Drugs used were dobutamine hydrochloride (Shionogi Pharmaceutical Co., Osaka, Japan), terbutaline sulphate and metoprolol tartrate (Fujisawa Pharmaceutical Co., Osaka, Japan) and ICI-118551 hydrochloride (ICI Pharmaceutical Division, Town, England).

Statistical analysis: Data are presented as the mean $\pm$ S.E. of data from 6 rats. The statistical significance of differences was assessed by Student's *t*-test.

Results

Secretory response of fluid from submandibular glands: Patterns of secretion of saliva elicited by dobutamine and terbutaline from the submandibular gland were similar when these drugs were administered alone, but differed when rats were also treated with antagonists. The salivary flow rates reached a maximal level 5–10 min after administration of either dobutamine or terbutaline at each dose tested (5, 10, 20 and 40 mg/kg) and decreased gradually thereafter. The flow rate measured 30 min after administration of dobutamine at doses of 5, 10, 20 and 40 mg/kg decreased by 75.7, 62.6, 46.4 and 39.4% from the maximum levels, whereas the flow rate 30 min after administration of terbutaline decreased by 47.2, 39.1, 47.2 and 49.1%, respectively (Fig. 1). However, when metoprolol was injected prior to administration of dobutamine at a dose of 40 mg/kg, the salivary flow rates reached maximal levels after 5–10 min with a dose of 0.2 mg/kg of metoprolol and after 15–20 min with a dose of 1.0 mg/kg. With a dose of 5 mg/kg of metoprolol, no maximum value was reached (Fig. 2A). When ICI-118551 was administered prior to dobutamine, flow rates reached maxi-

imum values after 5–10 min, as did those observed after administration of dobutamine alone (Fig. 2A). In contrast to these results, the time required to reach the maximum flow rate after administration of terbutaline was not changed by pretreatment with either metoprolol or ICI-118551 at the above doses (Fig. 2B). Total volumes of saliva secreted from the submandibular glands during the 30 min which followed administration of either dobutamine or terbutaline increased in a dose-dependent manner, and the volume of saliva elicited by dobutamine at doses of 10–40 mg/kg were significantly higher than those elicited by terbutaline, as shown by the ED₅₀ value, which was 8.7 mg/kg for dobutamine and 5.9 mg/kg for terbutaline (Fig. 3). The volumes of saliva elicited in response to either dobutamine or terbutaline at a dose of 40 mg/kg were 39.82 ± 2.60 and 21.15 ± 0.80 μ l/100 mg wet weight gland/30 min, respectively. After pretreatment with each antagonist at doses of 0.2, 1.0 and 5.0 mg/kg, the volumes of saliva elicited by dobutamine respectively decreased by 52.1, 56.6 and 87.0% with metoprolol and by 23.3, 29.1 and 35.6% with ICI-118551; and the volume of saliva elicited by terbutaline respectively decreased by 40.6, 52.4 and 86.3% with metoprolol and by 22.9,

Fig. 1. A comparison of flow rates of saliva elicited from rat submandibular glands by i.p. administration of dobutamine or terbutaline at different doses. Each point represents the mean $\pm$ S.E. of results from six animals.

Fig. 2. Effects of pretreatment with either metoprolol or ICI-118551 on the flow rates of saliva elicited from rat submandibular glands by administration of dobutamine (A) and terbutaline (B). Each antagonist was injected i.v. at a dose of 0.2, 1.0 and 5.0 mg/kg, 30 min prior to i.p. administration of each agonist at a dose of 40 mg/kg. Each value represents the mean $\pm$ S.E. of results from six animals.

29.4 and 59.2% with ICI-118551 (data not shown).

Concentration and total amounts of protein in saliva secreted from the submandibular gland: The concentration of protein in saliva secreted from the submandibular gland during the 30 min which followed the administration of dobutamine increased in a dose-dependent manner, whereas with terbutaline, the concentration of protein showed a tendency to

decrease with increasing doses of the drug. However, the concentrations of protein in saliva evoked by dobutamine were significantly lower than those evoked by terbutaline, at the doses of 5, 10 and 20 mg/kg, but not at 40 mg/kg. In addition, the total amounts of protein secreted from the submandibular glands in response to either dobutamine or terbutaline increased progressively with increasing doses, with an

especially sharp increase at doses from 10 to 20 mg/kg of dobutamine. However, the amounts of protein in saliva evoked by dobutamine at the dose of 5 mg/kg were significantly lower than those in saliva evoked by terbutaline (Table 1). When doses of 40 mg/kg of dobutamine were administered after pretreatment with metoprolol or ICI-118551

Fig. 3. Dose-response curve for the volume of saliva elicited from rat submandibular gland by i.p. administration of dobutamine or terbutaline. Total volume is represented as μ l of saliva secreted per 100 mg wet weight of the submandibular gland for the 30 min period which followed i.p. administration of dobutamine or terbutaline. Each point represents the mean $\pm$ S.E. of results from six animals. ** and *** indicate a significant difference from the results with dobutamine at $P < 0.01$ and $P < 0.001$, respectively.

at doses of 0.2, 1.0 and 5.0 mg/kg (i.v.), the concentration of protein in saliva evoked by dobutamine decreased as the doses of metoprolol increased, but was not changed significantly by pretreatment with ICI-118551. The amount of protein was decreased by 44.2, 60.2 and 91.0% by pretreatment with metoprolol at the respective doses and by 19.5, 38.3 and 38.3% by pretreatment of ICI-118551 at the respective doses (Table 2A). When terbutaline was administered at a dose of 40 mg/kg, the amounts of protein decreased by 14.8, 33.1 and 78.2% after pretreatment with metoprolol at doses of 0.2, 1.0 and 5.0 mg/kg, respectively and by 9.5 and 49.0% after pretreatment with ICI-118551 at doses of 1.0 and 5.0 mg/kg, respectively (Table 2B).

Electrophoretic profiles of the glycoproteins and proteins: The typical electrophoretic patterns of glycoproteins and proteins in the submandibular saliva elicited by i.p. administration of either dobutamine or terbutaline at a dose of 40 mg/kg are shown in Fig. 4. The patterns of glycoproteins and proteins did not differ significantly between dobutamine-evoked and terbutaline-evoked saliva. Furthermore, the patterns obtained at lower doses of either dobutamine or terbutaline (at 5, 10 and 20 mg/kg) were similar to those obtained at a dose of 40 mg/kg (data not shown). The glycoprotein profiles of dobutamine- and terbutaline-evoked saliva showed a heavily stained band I and two minor bands,

Table 1. A comparison of concentrations and total amounts of protein in saliva elicited from rat submandibular glands during the 30 min after i.p. administration of either dobutamine or terbutaline at different doses

Drug	Dose (mg/kg)	Protein conc. (mg/ml)	Protein secreted (mg/100 mg wet wt./30 min)
Dobutamine	5	23.86 $\pm$ 0.77	0.23 $\pm$ 0.04
	10	30.35 $\pm$ 1.50	0.67 $\pm$ 0.06
	20	34.95 $\pm$ 1.53	1.27 $\pm$ 0.14
	40	38.34 $\pm$ 2.43	1.51 $\pm$ 0.08
Terbutaline	5	46.90 $\pm$ 3.11***	0.42 $\pm$ 0.02**
	10	42.78 $\pm$ 2.55**	0.64 $\pm$ 0.09**
	20	42.94 $\pm$ 1.71**	0.81 $\pm$ 0.04
	40	41.52 $\pm$ 1.41	0.87 $\pm$ 0.02***

Each value represents the mean $\pm$ S.E. of results from six animals. ** and *** indicate a significant difference from the results with dobutamine at $P < 0.01$ and $P < 0.001$, respectively.

Table 2. Effects of pretreatment with either metoprolol or ICI-118551 on the concentrations and total amounts of protein in saliva elicited from rat submandibular glands by dobutamine (A) and by terbutaline (B)

Antagonist	Dose (mg/kg)	protein conc. (mg/ml)	protein secreted (mg/100 mg wet wt./30 min)
A. Dobutamine			
—	—	38.34±2.43	1.51±0.08
Metoprolol	0.2	44.06±2.13	0.84±0.09***
	1	35.11±2.77	0.60±0.07***
	5	22.12±2.78***	0.14±0.03***
	0.2	40.05±3.18	1.21±0.09*
ICI 118551	1	32.91±1.93	0.93±0.07***
	5	36.48±1.61	0.93±0.05***
B. Terbutaline			
—	—	41.52±1.41	0.87±0.02
Metoprolol	0.2	59.64±3.59***	0.75±0.06
	1	57.98±2.45***	0.59±0.04***
	5	64.25±5.25**	0.19±0.03***
	0.2	53.37±3.59*	0.88±0.08
ICI 118551	1	53.36±5.13	0.79±0.09
	5	49.33±4.87	0.45±0.08***

Each antagonist was injected i.v. at a dose of 0.2, 1.0 and 5.0 mg/kg, 30 min prior to i.p. administration of dobutamine and terbutaline at a dose of 40 mg/kg. Each value represents the mean±S.E. of results from six animals, five animals for (*). *, ** and *** indicate a significant difference from the results with control at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

III and IV. The pattern of glycoproteins from secretory segments isolated from the submandibular gland of the normal rat showed that the acinar segments contain one major band, band (I), and three minor bands, bands (II), (III) and (IV), whereas the granular tubule segments contain one major band, band (III), and two minor bands, band (I) and band (II), as reported previously (14) (data not shown). When dobutamine- or terbutaline-evoked saliva were co-electrophoresed with samples of the acinus or samples of the granular tubules, the main bands I and IV of the saliva were electrophoretically identical with the main bands (I) and

(IV) in the acinar samples, whereas band III was electrophoretically identical to the major band (III) in the granular tubules. In the figure, the PAS-positive bands are indicated by an arrow above the corresponding Coomassie brilliant blue-stained bands. The protein band corresponding to glycoprotein band I is hardly stained with Coomassie blue, whereas the proteins which correspond to glycoprotein bands III and IV were strongly stained (Fig. 4B). The apparent molecular weights of proteins in bands I, III and IV are 130K, 31K and 21.5K daltons, as reported previously (14). When rats were pretreated with metoprolol at a dose of 5 mg/kg, dobu-

Fig. 4. Typical densitometric scans of glycoprotein (A) and proteins (B) of submandibular saliva. Metoprolol at a dose of 5 mg/kg was injected i.v. 30 min prior to i.p. administration of dobutamine or terbutaline at a dose of 40 mg/kg. Samples containing 0.5 and 0.25 µg of protein were applied to gels. After electrophoresis, gels were stained with PAS or Coomassie Blue. Electrophoresis was performed as described in Materials and Methods. PAS-positive bands are indicated by an arrow above the corresponding Coomassie Blue-stained band.

A

Dobutamine

Terbutaline

Dobutamine
+
Metoprolol Terbutaline
+
Metoprolol **B**

Dobutamine

Terbutaline

Dobutamine
+
Metoprolol Terbutaline
+
Metoprolol

tamine at a dose of 40 mg/kg decreased the intensity of glycoprotein bands I and IV (Fig. 4A), but such a result was not observed with metoprolol at the doses of 0.2 and 1.0 mg/kg (data not shown). When ICI-118551 was administered prior to dobutamine, the patterns were similar to those observed after treatment with dobutamine alone (data not shown). On the other hand, the patterns of terbutaline-evoked saliva were not altered by pretreatment with metoprolol (Fig. 4) or ICI-118551 (data not shown). Furthermore, electrophoretic patterns of proteins stained with Coomassie blue in dobutamine- and terbutaline-evoked saliva did not change after pretreatment with metoprolol (Fig. 4) or ICI-118551 (data not shown).

Discussion

In the present study, the ED₅₀ value with respect to secretion of saliva from the submandibular gland of rats was 8.7 mg/kg for dobutamine and 5.9 mg/kg for terbutaline. A difference between dobutamine and terbutaline has also been observed in the secretory potency for the salivation from the submandibular gland of rats (4). On the other hand, the concentration of protein in terbutaline-evoked saliva was significantly higher than that of dobutamine-evoked saliva when drugs were administered at doses of 5, 10 and 20 mg/kg, respectively, but no significant difference was found at the dose of 40 mg/kg (Table 1). In fact, terbutaline evoked a small volume of saliva rich in protein. Thus, these results suggest that terbutaline administered at small doses stimulates β_2 -receptors, as generally recognized, but at large doses, it may stimulate both β_1 -receptors and β_2 -receptors.

To estimate receptors mediating these reactions, three doses of metoprolol, which has been characterized as a selective β_1 -antagonist (22), and ICI-118551, which has been characterized as a selective β_2 -antagonist (23, 24), were applied before 40 mg/kg of dobutamine or terbutaline was administered. Pretreatment with metoprolol inhibited secretion of fluid (Fig. 2A) and protein (Table 2A) induced by dobutamine in a dose-dependent manner. On the other hand, the inhibitory effect of ICI-118551 was slight and considerably lower than that of me-

toprolol (Fig. 2A, Table 2A). This result may be attributed to a low level of activity toward β_2 -adrenoceptors of dobutamine, which was suggested by observations of the relaxation of vascular smooth muscle (25–27) and the adenylate cyclase or GTPase activity in erythrocyte membranes of the turkey and frog (28). Furthermore, pretreatment with metoprolol more strongly inhibited the secretion of fluid and protein induced by terbutaline than pretreatment with ICI-118551 (Fig. 2B, Table 2B). The present data suggests that terbutaline probably activates β_1 -adrenoceptors, rather than β_2 -adrenoceptors. This conclusion is consistent with the reports that secretion of either fluid or calcium from submandibular gland (3, 4) and enlargement of the parotid and submandibular gland (26) induced by administration of terbutaline were inhibited more effectively by the selective β_1 -antagonist atenolol than the selective β_2 -antagonist butoxamine.

On the other hand, the concentration of protein in dobutamine evoked saliva were reduced in a dose-dependent manner by pretreatment with metoprolol, but were not changed significantly by pretreatment with ICI-118551 (Table 2A). In contrast, the concentrations of protein in terbutaline-evoked saliva were increased more significantly by pretreatment with metoprolol than by pretreatment of ICI-118551 (Table 2B). This increase may be produced by mediation of β_2 -receptors because of a blockade of β_1 -receptors. Therefore, secretion of saliva rich in protein may be caused by activation of β_2 -receptors rather than β_1 -receptors.

The present data thus show that β -adrenoceptor mediated secretion of saliva and protein from rat submandibular glands would be mainly due to receptors of the β_1 -subtype. This speculation is consistent with the results on radioligand binding (29, 30), which indicate that the β -adrenoceptors of the rat submandibular glands are predominantly of the β_1 -type. However, our data suggests that the possibility that β_2 -receptors may exist in the submandibular gland cannot be neglected, since ICI-118551 has partially inhibitory effects on terbutaline. It is not known whether secretion of saliva and protein induced by terbutaline at small doses is

caused only by activation of β_2 -receptors.

Moreover, in this study, the action of dobutamine and terbutaline on the secretory cells of the submandibular gland was confirmed by estimations of marker species of glycoproteins, which are contained in the secretory segments and detected in drug-evoked saliva. The administration of dobutamine or terbutaline at doses of 5, 10, 20 and 40 mg/kg elicited the secretion of the glycoproteins designated as bands I and IV, which are characteristically contained in acinar cells (Fig. 4). However, the intensities of both bands were considerably higher than those found by administration of choline esters (31); these phenomena indicate that both dobutamine and terbutaline acts on acinar cells more strongly than choline esters. On the other hand, when rats were pretreated with metoprolol at a dose of 5 mg/kg, dobutamine at a dose of 40 mg/kg produced a decrease in intensity of glycoprotein bands I and IV, although such a change was not observed after pretreatment with ICI-118551 (Fig. 4). This decrease in band I may be caused by a blockade of the β_1 -adrenoceptors of the acinus. On the other hand, when administered with a prior dose of 5 mg/kg of ICI-118551 or metoprolol, terbutaline, at a dose of 40 mg/kg, generated profiles of glycoproteins which were similar to those found in saliva evoked by terbutaline alone. Abe et al. (3) have shown that the electrophoretic patterns of proteins in saliva elicited from submandibular glands of rats by terbutaline were similar to those observed after treatment with isoproterenol, and these patterns were not altered by pretreatment with metoprolol or ICI-118551. Our findings are in agreement with these results; that is, the electrophoretic patterns were similar in dobutamine- and terbutaline-evoked saliva, and the patterns were not altered by pretreatment with metoprolol or ICI-118551.

In conclusion, the present study showed that the submandibular gland of the rat contains both β_1 - and β_2 -adrenoceptors and that the β -receptors, that mediate secretion of glycoprotein from the acinus and fluid from the gland are mainly of the β_1 -subtype.

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