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β Adrenergic and Muscarinic Receptor Densities of Rat Submandibular Main Duct (42520)

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Abstract. [^{3}H]DHA binding studies show that main duct of rat submandibular gland has both β_1 and β_2 adrenoceptors, with the percentages of each being 69 and 31%, respectively, whereas whole submandibular gland has 90% β_1 and 10% β_2 adrenoceptors. Muscarinic receptors of main duct are 25% less than that of whole submandibular gland. © 1987 Society for Experimental Biology and Medicine.

Evidence based on radioligand binding in salivary glands (1) as well as that based on the functional responses of salivary glands to specific β_1 and β_2 adrenergic agonists (2, 3) indicates that the β adrenoceptors of rat salivary glands are mainly of the β_1 subtype. However, a few reports indicate that β_2 adrenoceptors may also be present (4, 5). Data obtained using terbutaline and dobutamine, specific β_2 and β_1 agonists, on perfused main duct of submandibular gland, support the view that electrolyte transport in the main duct is mediated by both β_1 and β_2 adrenoceptors (6). The present work on radioligand binding of receptors in main duct was undertaken to establish that both kinds of β adrenoceptors are present in the main duct.

Materials and Methods. Female Long–Evans rats, 4 months of age, were maintained on solid lab chow and water *ad libitum* until 18 hr before sacrifice. Under anesthesia induced by ip administration of sodium pentobarbital (50 mg/kg), the paired main ducts of the submandibular gland were removed from 40 rats; pooled samples of 10 ducts (from 5 rats) were removed, weighed rapidly on a torsion balance, and then placed in 10 m*M* Tris–HCl buffer for subsequent membrane isolation and assays for receptor densities. The whole submandibular glands were also removed and individually placed in 10 m*M* Tris–HCl buffer for subsequent plasma membrane isolation.

For preparation of plasma membrane fractions, submandibular gland or isolated duct cell homogenates were centrifuged at 800*g* for 5 min to remove connective tissue, unlysed cells, and nuclei. The supernatant was centrifuged a second time at 20,000*g* for 30 min at 4°C. This fraction was highly enriched for the plasma membrane and contained very little contaminating intracellular membrane as determined by appropriate membrane enzyme markers (7). While total membrane activity for the Golgi enzyme, 4β-galactosyltransferase was, for six rats, 0.25 ± 0.03 nmole/min/mg protein, plasma membrane enzyme activity was 0.01 nmole/min/mg protein, indicating little contamination from internal membrane fractions. Endoplasmic reticulum membranes from Golgi and secretory vesicles were isolated by recentrifugation of the above supernatant at 150,000*g* for 1 hr. Endoplasmic reticulum membranes demonstrated little Golgi-specific enzyme activity while the supernatant recovered from this centrifugation step contained a substantial galactosyltransferase enrichment (number of rats, for each mean, 6, 0.03 ± 0.01, and 2.89 ± 0.15 nmol/min/mg protein, respectively). The pellet containing the membrane fraction was resuspended in 100 vol of 10 m*M* Tris–HCl buffer, pH 7.6, containing 5 m*M* $MgCl_2$ and 100 μM dithiothreitol. Membranes were resuspended by a combination of vortex vibration followed by Dounce homogenization. Protein concentrations were subsequently determined by modification of the Lowry protein assay using bovine serum albumin as standard (8). Binding of [^{3}H]-quinclidinylbenzilate ([^{3}H]QNB) and [^{3}H]-dihydroalprenolol ([^{3}H]DHA) (Amersham Corp., Arlington Heights, IL) were linearly dependent on membrane concentration within this dilution of both the submandibular duct cells and whole glands. Binding assays were performed in duplicate using 1.0 ml of diluted membrane and 1.0 n*M* [^{3}H]QNB or [^{3}H]DHA concentrations of 0.1 and 2.0 n*M*. Binding of

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labeled antagonists was saturable at 0.5 n*M*. The reaction mixture was incubated for 30 min at 37°C, and terminated by the addition of 3 ml ice-cold 0.9% NaCl. Quantitation of binding was performed by precipitation of membranes from the above slurry onto glass fiber filters, washed three times with 5 vol of cold PBS, and counted for radioactivity by liquid scintillation. Nonspecific binding for muscarinic receptors was determined by the inclusion of 1.0 μM atropine (Sigma Chemical Co., St. Louis, MO) 10 min prior to the addition of labeled QNB (10 μM). Propranolol (Sigma Chemical Co.) was used to determine nonspecific binding in experiments using [^{3}H]DHA.

Results. Receptor assays of duct cells and whole submandibular glands were performed at saturating concentrations of [^{3}H]QNB (9). The data in Table I show [^{3}H]DHA binding of membranes of main duct of submandibular gland, alone and in the presence of the selective β_1 and β_2 adrenergic antagonists, atenolol and butoxamine. Data from whole submandibular gland obtained under the same experimental conditions are also presented in Table I. DHA binding of whole submandibular gland in the presence of atenolol was 10%, and in the presence of butoxamine was 90%; however, DHP binding of ductal membranes in the presence of atenolol was 31% and in the presence of butoxamine it was 69%. There were also differences between submandibular whole gland and main duct in muscarinic receptors. Density based on [^{3}H]QNB binding was, for main duct, decreased 25% from that of whole submandibular gland.

Discussion. Present radioligand binding studies generally confirm results based on physiological responses to the selective β_1 and β_2 adrenergic agonists and show that the predominant β adrenoceptor in the whole submandibular gland is β_1, but the main submandibular duct has large (31%) numbers of β_2 as well as β_1 adrenoceptors. It now also appears probable that any β_2 adrenoceptors present in the whole gland may reflect the ductal component of the gland. The present data thus provide an explanation for the differences between submandibular duct and whole submandibular gland in response to terbutaline and dobutamine. Thus, transport of potassium in main duct is mediated by β_2 as well as β_1 adrenoceptors (6); however, growth responses of the whole gland (2) and secretion of calcium, e.g., (3), appear to be mediated predominantly by β_1 adrenoceptors. These functions that are β_1 mediated have been associated with acinar cells; the radioligand binding data of whole gland thus reflect mainly the acinar component, with β_1 adrenoceptors principally evident, whereas ducts have both β_1 and β_2 adrenoceptors. While the main duct has fewer muscarinic receptors than the whole gland, their number in main duct is nonetheless high; activation of these receptors accounts for the changes in Na and K transport during stimulation of the parasympathetic nerve to submandibular gland (10).

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TABLE I. PROPERTIES OF MEMBRANES OBTAINED FROM RAT SUBMANDIBULAR MAIN DUCT AND WHOLE GLAND

	Receptor density			
	β Adrenergic [^{3}H]DHA binding (fmole/mg membrane protein)			Muscarinic [^{3}H]QNB binding (fmole/mg membrane protein)
Submandibular	−Antag.	+Atenolol	+Butoxamine	
Gland (6)[a]	69.6 ± 0.8	7.4 ± 0.3	62.7 ± 1.4	141 ± 0.7
Ducts (80)[b]	38.2 ± 1.9	11.7 ± 1.3	26.2 ± 1.9	106 ± 0.4

[a] Value refers to number of rats.

[b] Value represents the paired ducts from 40 rats; for each determination 10 ducts (5 rats) were pooled; therefore, the mean represents the mean of eight sets of pooled ducts.

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