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K^+ RELEASE FROM RAT PAROTID CELLS: AN α_1-ADRENERGIC MEDIATED EVENT

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Abstract—To characterize α-adrenergic receptors in rat parotid gland tissue, 9,10-[9,10-^{3}H(N)]-dihydro-α-ergocryptine ([^{3}H]DHE) and [^{3}H]prazosin binding to membranes and stimulated K^+ release from parotid cell aggregates were examined. Prazosin (selective α_1-adrenergic antagonist), displacement of [^{3}H]DHE binding from parotid membranes was biphasic, indicating the presence of both α_1- and α_2-adrenergic receptors. The numbers of α_1- and α_2-receptors were about equal. α_1-Adrenergic receptors were further studied by [^{3}H]prazosin binding. [^{3}H]Prazosin binding was a rapid, reversible, saturable and stereospecific process, with high affinity ($K_D = 0.38$ nM) and low capacity ($B_{max} = 380$ fmoles ligand bound/g tissue, 10.1 fmoles/mg protein) as determined by Scatchard analysis. The characteristics of [^{3}H]prazosin binding were in good agreement with those of catecholamine-stimulated K^+ release, suggesting that K^+ release from rat parotid gland cells is an α_1-adrenergic mediated effect.

Recent progress in α-adrenergic receptor studies has revealed the existence of at least two subtypes of α-adrenergic receptors [1–5]. α-Adrenergic receptors were initially subdivided on an anatomic basis [1, 2] into pre- and post-synaptic α-adrenergic receptors. Subsequently, α-adrenergic receptors with pre-synaptic characteristics have been found on non-neuronal tissue such as human platelets [5, 6]. It thus seemed preferable to use the nomenclature of α_1 and α_2 for the subtypes of α-adrenergic receptors on the basis of their pharmacologic characteristics in the same way as β_1 and β_2 are used for the subtypes of β-receptors [3].

Several reports have examined α-adrenergic mediation of K^+ release in various rat salivary glands [7–11]. In the submandibular gland, a mixed seromucinous tissue, the existence of both subtypes of α-adrenergic receptors has been demonstrated [4, 6, 12, 13]. Though the role of each receptor subtype in regulating K^+ release from the submandibular gland has not been clearly established, the work of Arnett and Davis [12] seems to suggest that the α_2-receptor may be particularly important. In addition, Davis and Maury [10] reported that clonidine, believed to have a selectivity for the α_2-receptors in some tissues [3, 4], could block (−)-epinephrine-stimulated K^+ release in dispersed rat parotid cells. However, in this latter serous-type gland, a tissue frequently utilized in studies of exocrine gland function [14], there has been no direct examination of α-adrenergic subtype distributions present nor of their particular role in K^+ release.

The present report is the first attempt to distinguish between the two α-adrenergic receptor subtypes in rat parotid gland and to examine their roles in K^+ release from that tissue. Our data clearly indicate that, although α_1- and α_2-adrenergic receptors are present in approximately equal amounts in rat parotid gland, it is the α_1-receptor subtype that mediates K^+ release.

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MATERIALS AND METHODS

Animals. Three- to six-month-old male Wistar rats were used in this study. All animals were obtained from the Gerontology Research Center (NIA) colony. Animals were maintained on NIH Purina laboratory chow and water *ad lib.* until killed.

Chemicals. Chromatographically purified collagenase (type CLSPA Lot No. 50E373, sp. act. 397 units/mg) was purchased from the Worthington Biochemical Corp., Frechold, NJ. Bovine testicular hyaluronidase (type 1-S, sp. act. 270 NF units/mg), bovine serum albumin (Fraction V), (−)-epinephrine bitartrate, (−)-norepinephrine bitartrate, (−)-phenylephrine hydrochloride, (±)-normetanephrine hydrochloride and yohimbine hydrochloride were obtained from the Sigma Chemical Co., St. Louis, MO. Phenoxybenzamine hydrochloride and (±)-propranolol hydrochloride were purchased from Smith Kline & French Laboratories, Philadelphia, PA, and Ayerst Laboratories, New York, NY, respectively. Prazosin hydrochloride, phentolamine mesylate, (+)-isomers of epinephrine and norepinephrine bitartrate were gifts from Pfizer Laboratories Division, New York, NY, CIBA Pharmaceutical Co., Summit, NJ, and Sterling-Winthrop Research Institute, Rensselaer, NY, respectively.

All other chemicals used were the highest grade commercially available.

Radioactive ligands. 9,10-[9,10-^{3}H(N)]-Dihydro-α-ergocryptine ([^{3}H]DHE, sp. act. 30.9 Ci/mmole) was obtained from the New England Nuclear Corp., Boston, MA. [^{3}H]Prazosin (sp. act. 28 Ci/mmole)

was purchased from the Amersham Corp., Arlington Heights, IL.

[^{3}H]DHE and [^{3}H]prazosin were stored at −20° in 100% ethanol and 50% ethanol containing 5 mM hydrochloric acid respectively. Both radioactive ligands were diluted with 10% ethanol containing 5 mM hydrochloric acid less than 30 min before the start of experiments.

Preparation of parotid cell aggregates. All rats were killed between 9.30 and 10.00 a.m. by bleeding via the abdominal aorta after stunning by a blow to the head. Parotid glands were removed quickly, trimmed of connective tissue, fat tissue and lymph nodes, and minced finely with iris scissors.

Enzymatically dispersed parotid cell aggregates were obtained by a slight modification of the method of Strittmatter *et al.* [9, 15]. Briefly, tissue from one rat was suspended in 5 ml of enzyme solution: collagenase (96 units/ml) and bovine testicular hyaluronidase (0.19 mg/ml) in Hanks' balanced salt solution* adjusted to pH 7.4 at 20° after full oxygenation by bubbling with 95% O_2–5% CO_2 for at least 30 min. Tissue was incubated for 60 min at 37° in a Dubnoff metabolic shaker. Incubations were gassed every 15 min with 95% O_2–5% CO_2 and tissue was dispersed by gentle pipetting. The pH of the solution did not change appreciably during the incubation.

Preparation of membranes from parotid cell aggregates. Following incubation with enzymes, cell aggregates were washed twice with 5 ml of 4% bovine serum albumin in Hanks' balanced salt solution and twice with ice-cold 50 mM Tris buffer (pH 7.4 at 20°). Finally, cell aggregates were suspended in 20 vol. (w/v) of ice-cold 50 mM Tris buffer and homogenized with a Polytron PT-10 homogenizer (Brinkmann Instruments, Westbury, NY) for three 10-sec bursts at setting 5. The homogenate was filtered through four layers of gauze. The resultant filtrate was centrifuged twice for 10 min at 48,000 *g* with resuspension of the pellet in 20 vol. of fresh buffer between spins. The final pellet was rehomogenized with a Polytron PT-10 homogenizer for 10 sec in 50 vol. of ice-cold 50 mM Tris buffer, and used directly in the binding studies.

[^{3}H]DHE or [^{3}H]prazosin binding assay. The standard [^{3}H]DHE or [^{3}H]prazosin binding assay contained 1 ml of membrane suspension (~0.8 mg of protein) in 50 mM Tris buffer (containing membranes from 20 mg of parotid cell aggregates), 0.8 mM ascorbate, 3 mM catechol, 1 μM (±)-propranolol and radioactive ligand in a final volume of 1.02 ml. These components were included because they have been reported to reduce non-specific binding of catecholamines [16–18], but in the present system have no appreciable effect on either specific or non-specific binding. The incubation was carried out in 17 × 100 mm polypropylene tubes (Falcon, Oxnard, CA) at 20°, for 60 min in the dark for [^{3}H]DHE binding and for 30 min in the room light for [^{3}H]prazosin binding, respectively, unless otherwise indicated. Following incubation to equilibrium, tubes were filtered under vacuum through Whatman GF/C glass-fiber filters with three 5-ml washes of 50 mM Tris buffer. Filters were dried at 60° for 1 hr, placed in 4 ml of ACS scintillation fluid (Amersham), and counted in a Packard Tri-Carb liquid scintillation counter (model 574) with 38% efficiency.

Specific binding of radioligands was defined as the difference between total binding and non-specific binding measured in the presence of 10^{-5} M phentolamine and was 100% reversible. Specific binding of both ligands was also completely displaceable by 10^{-5} epinephrine and norepinephrine. In addition, specific binding was unaffected when assays were performed in the presence of 140 mM NaCl (intact cell conditions).

Measurement of K^+ release from paratid cell aggregates. Following incubation with enzymes, cell aggregates were washed twice with 5 ml of 4% bovine serum albumin in Hanks' balanced salt solution containing 3.1 m-equiv./l of K^+ and twice with 5 ml of 0.022% bovine serum albumin in Hanks' balanced salt solution containing 3.1 m-equiv./l of K^+ (incubation medium). Cell aggregates were divided into several portions, resuspended in the incubation medium (4.5 ml/gland), and incubated for 30 min at 37° with constant shaking. Cell aggregates were centrifuged at 40 *g* for 15 sec, resuspended in 360 μl of the incubation medium, and incubated for an additional 10 min. Then, catecholamines, prepared just before use, were added to the tubes. When antagonists were used in the experiments, cell aggregates were incubated with antagonists for 10 min prior to the addition of catecholamine. Total incubation volume was 400 μl. Incubations were carried out for 45 sec at 37° with constant shaking, and centrifuged at 780 *g* for 10 sec. One hundred microliters of incubation medium supernatant fluid was collected at 60 sec after the catecholamine addition. Seven hundred microliters of distilled water was added to the remaining cell aggregate suspension, and the suspension was then homogenized with a Polytron PT-10 homogenizer for 10 sec at setting 5. The resultant homogenates were centrifuged at 780 *g* for 10 min. The K^+ concentrations in the incubation medium supernatant fractions and the supernatant fractions of homogenates were assayed by atomic absorption (Perkin–Elmer 306) after appropriate dilution with distilled water [8]. This assay technique measures the incremental increase in extracellular K^+ in response to catecholamine addition.

Intracellular and released K^+ amount and, then, percent release of K^+ were calculated. K^+ release stimulated by 10^{-5} M (−)-epinephrine was 24 ± 7% (mean ± S.E., N = 5) of total intracellular K^+.

Calculation of K_d, K_i and EC_{50} values. The ability of adrenergic agents to displace radioactive ligand from membrane binding sites (K_D) and the ability of adrenergic antagonists to inhibit (−)-epinephrine-stimulated K^+ release (K_i) were calculated according to Cheng and Prusoff [19]. The EC_{50} value of catecholamines in stimulating K^+ release was determined by replotting dose–response curves on probit paper and determining the concentration causing a half-maximal effect.

Measurements of protein concentration. Protein concentration was determined according to the

* Hanks' balanced salt solution contained 136.7 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4$–$7H_2O$, 1.3 mg $CaCl_2$, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.6 mM dextrose, and 4.2 mM $NaHCO_3$.

method of Lowry *et al.* [20], employing bovine serum albumin as a standard.

RESULTS

Binding of [^{3}H]*DHE to parotid membranes.* Binding of [^{3}H]DHE to parotid membranes was rapid, reaching equilibrium by 60 min at 20° (data not shown). Specific [^{3}H]DHE binding represented 30–40% of total binding at 1 nM [^{3}H]DHE. Specific binding was defined as the difference between binding of the radioligand in the absence and presence of 10 μM phentolamine.

In saturation studies [^{3}H]DHE appeared to bind to a single population of sites on parotid membranes with a dissociation constant, K_D, of 2.1 ± 0.3 nM and a maximum binding capacity, B_{max}, of 1.2 ± 0.4 pmoles ligand bound/g tissue (32 ± 11 fmoles ligand bound/mg protein) (N = 3) when data were analyzed according to the method of Scatchard [21]. Figure 1 shows biphasic displacement curves of [^{3}H]DHE bound to parotid membranes with both prazosin, a selective α_1-adrenergic antagonist [22–24], and yohimbine, a selective α_2-adrenergic antagonist [23]. These results suggest the presence of both α_1- and α_2-adrenergic receptors in parotid membranes. The proportion of subtypes appears about equal. α_1- and α_2-adrenergic receptors have been assayed separately by measuring [^{3}H]DHE binding in the presence of yohimbine and prazosin according to Miach *et al.* [25]. However, the titration of yohimbine displacement of [^{3}H]DHE binding in parotid membranes (Fig. 1B) is not as clear as that reported in brain tissue [25]. This result suggests difficulties would follow measuring directly α_1-adrenergic receptors in parotid membrane with [^{3}H]DHE as a radioligand.

Binding of [^{3}H]*prazosin to parotid membranes.* Binding of the selective α_1-adrenergic antagonist,

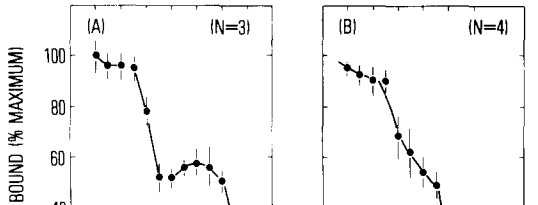

Fig. 1. Displacement of [^{3}H]DHE binding from rat parotid membranes by prazosin (A) and yohimbine (B). Parotid membranes obtained from 20 mg of the original wet weight of parotid cell aggregates were incubated with 1 nM [^{3}H]DHE in the presence and absence of various concentrations of the specific α_1-adrenergic antagonist prazosin (A) or the specific α_2-adrenergic antagonist yohimbine (B) at 20° for 60 min. The details of the experimental conditions are described in Materials and Methods. Each point is the mean ± S.E. of the results obtained from three (A) or four (B) experiments. Parotid membranes were prepared from two rats in each experiment. Triplicate determinations were performed.

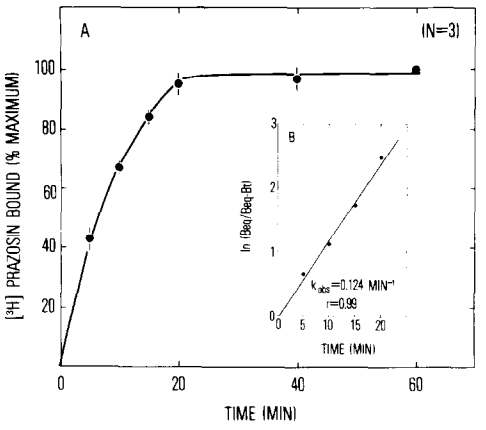

Fig. 2. Time course of specific binding of [^{3}H]prazosin to rat parotid membranes. (A) Binding of [^{3}H]prazosin as a function of time. Specifically bound [^{3}H]prazosin was determined at the various time points indicated in the figure as described in Materials and Methods. Maximum binding refers to the amount of [^{3}H]prazosin specifically bound at 60 min. Each point is the mean ± S.E. of results obtained from three experiments. Parotid membranes were prepared from two rats in each experiment. Triplicate determinations were performed. (B) Pseudo first-order rate plot of specific binding of [^{3}H]prazosin. Data were taken from the mean values presented in Fig. 2A. B_{eq} = concentration of specifically bound [^{3}H]prazosin at equilibrium. B_t = concentration of bound [^{3}H]prazosin at the time, t. The slope of the line determined by linear regression analysis is an estimate of the pseudo first-order association rate constant, k_{obs}, at 20°.

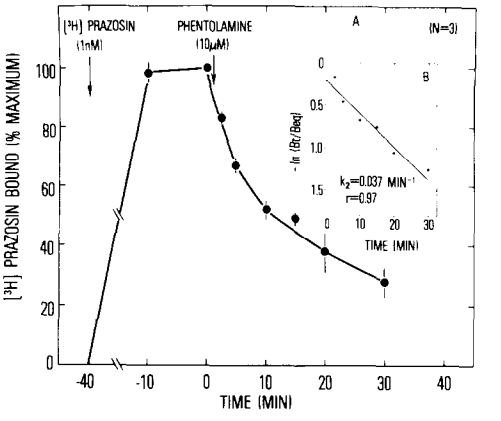

Fig. 3. Dissociation of [^{3}H]prazosin from rat parotid membranes. (A) Dissociation of [^{3}H]prazosin as a function of time. Excess amount of phentolamine (10 μM) was added to a membrane suspension preincubated with 1 nM [^{3}H]prazosin for 40 min at "zero time", and specifically bound [^{3}H]prazosin was determined at various time points indicated in the figure, as described in Materials and Methods. Maximum binding refers to the concentration of [^{3}H]prazosin specifically bound at equilibrium, just prior to the addition of phentolamine specifically at time "zero". Each point is the mean ± S.E. of results obtained from three experiments. Parotid membranes were prepared from two rats in each experiment. Triplicate determinations were performed. (B) First-order plot of dissociation of [^{3}H]prazosin from parotid membranes. Data were taken from the mean values presented in Fig. 3A, and calculated similarly to those in Fig. 2B. The slope of the line determined by linear regression analysis is an estimate of the first-order dissociation rate constant, k_2, at 20°.

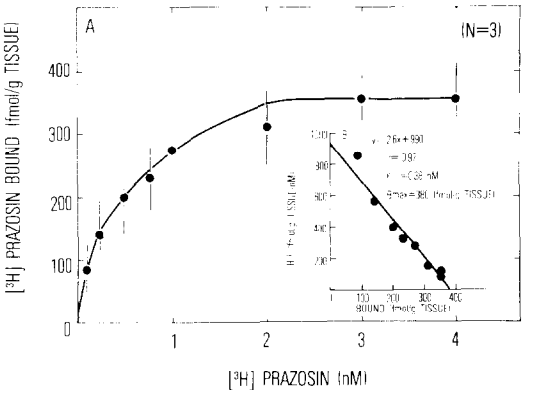

Fig. 4. [^{3}H]Prazosin binding to rat parotid membrane as a function of radioactive ligand concentration. (A) Specific binding as a function of [^{3}H]prazosin concentration. Specifically bound [^{3}H]prazosin was determined at various concentrations of the radioligand as described in Materials and Methods. Each point is the mean ± S.E. of results obtained from three experiments. Parotid membranes were prepared from two rats in each experiment. Triplicate determinations were performed. (B) Scatchard plot of specific [^{3}H]prazosin binding to parotid membranes. Data were taken from the mean values presented in Fig. 4A. The slope of the plot, $-1/K_D$, was determined by linear regression analysis and the maximum binding capacity, B_{max}, was computed from the intercept of the plot with the abscissa according to Scatchard [21]. This analysis gave a K_D value of 0.38 nM and a B_{max} of 380 fmoles ligand bound/g tissue. Alternatively, when data from three experiments were analyzed individually, a K_D value of 0.78 ± 0.32 and a B_{max} value of 427 ± 41 fmoles ligand bound/g tissue (13.2 ± 1.2 fmoles/mg protein) were obtained. Actual counts per minute in the 1 nM samples were 200–300 total and 100–150 specifically bound.

[^{3}H]prazosin, to parotid membranes was examined. In parotid membranes, specific [^{3}H]prazosin binding represented 40–60% of total binding at 1 nM [^{3}H]prazosin. Binding of [^{3}H]prazosin to parotid membranes was rapid, reaching equilibrium within 30 min at 30° (Fig. 2A). Kinetic data* from Fig. 2A can be used to calculate the true second-order association rate constant, k_1.

With 1 nM [^{3}H]prazosin, a pseudo first-order association rate constant, k_{obs}, of 0.124 min^{-1} was determined (Fig. 2B) from the data of Fig. 2A. Binding was rapidly reversible, with a k_2 of 0.037 min^{-1} (Fig. 3, A and B). From these data, k_1 was determined to be 8.7×10^7 M^{-1} min^{-1}, and K_D was determined to be 0.42 nM.

Alternatively, when data from three experiments were calculated separately, and averaged, the k_{obs} values were 0.118 ± 0.011 min^{-1} ($r = 0.97 \pm 0.02$), and the k_2 value was 0.038 ± 0.022 min^{-1} ($r = 0.96 \pm 0.01$). From these data k_1 and K_D were computed as 8.0×10^7 M^{-1} min^{-1} and 0.48 nM respectively.

Incubation of parotid membranes with increasing concentrations of [^{3}H]prazosin (0.1 to 4 nM) for 30 min at 20° showed that specific binding was a saturable process with approximately 360 fmoles ligand bound/g tissue (9.6 fmoles/mg protein) (Fig. 4A). Half-maximal saturation occurred at 0.4 to 0.5 nM, providing an estimate of a K_D values for [^{3}H]prazosin binding. For more accurate determination of both K_D and B_{max}, the results in Fig. 4A were analyzed according to the method of Scatchard (Fig. 4B). This analysis gave a K_D value of 0.38 nM and a B_{max} value of 380 fmoles ligand bound/g tissue (10.1 fmoles/mg protein).

The ability of a variety of adrenergic antagonists and agonists to displace [^{3}H]prazosin binding from membranes is shown in Figs. 5 and 6 respectively. The potency of the adrenergic antagonists and agonists in displacing [^{3}H]prazosin binding was prazosin ≥ phenoxybenzamine ≫ yohimbine ≥ (±) propranolol (Fig. 5), and (−)-epinephrine ≥ (−)-norepinephrine > (−)-phenylephrine > (±)-normetanephrine (Fig. 6) respectively. Displacement of [^{3}H]prazosin was stereospecific, the (−)-isomers of epinephrine and norepinephrine being more potent than the (+)-isomers (Fig. 6).

The K_D value of nonradioactive prazosin (2 nM) was in fairly good agreement with that determined with the radioligand: 0.38 nM and 0.56 nM, calculated from the saturation and kinetic binding studies respectively.

K$^+$ release from parotid cell aggregates. (−)-Epinephrine stimulation of K$^+$ release from rat parotid

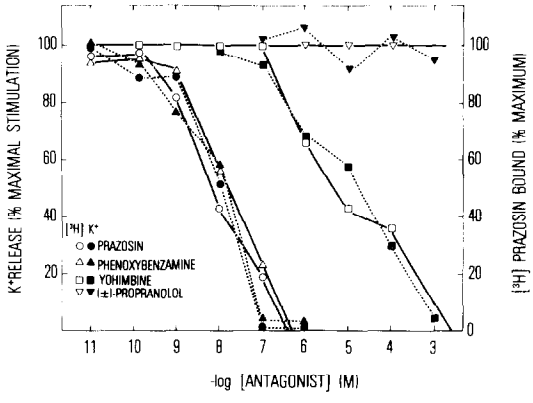

Fig 5. Displacement of [^{3}H]prazosin binding from rat parotid membranes and inhibition of the (−)-epinephrine-stimulated K$^+$ release from parotid cell aggregates by adrenergic antagonists. Right ordinate: Displacement of [^{3}H]prazosin binding. Parotid membranes were incubated at 20° for 30 min with 1 nM [^{3}H]prazosin and various concentrations of prazosin (○), phenoxybenzamine (△), yohimbine (□) and (±)-propranolol (▽). Maximum binding refers to the amount of [^{3}H]prazosin specifically bound in the absence of any antagonist. Each point is the mean of two experiments performed with triplicate determinations. Left ordinate: Inhibition of (−)-epinephrine-stimulated K$^+$ release. (−)-Epinephrine (10 μM) was added to parotid cell aggregate suspensions preincubated with various concentrations of antagonists, mentioned above (filled symbols), for 10 min at 37°. K$^+$ release, after the (−)-epinephrine addition, was determined as described in Materials and Methods. Maximum stimulation refers to K$^+$ release in the presence of 10 μM (−)-epinephrine, but in the absence of any antagonist. Each point is the mean of two to three experiments performed with quadruplicate determinations.

* Data from kinetic studies were analyzed according to L. T. Williams and R. J. Lefkowitz, *Receptor Binding Studies in Adrenergic Pharmacology*, p. 27, Raven Press, New York (1978).

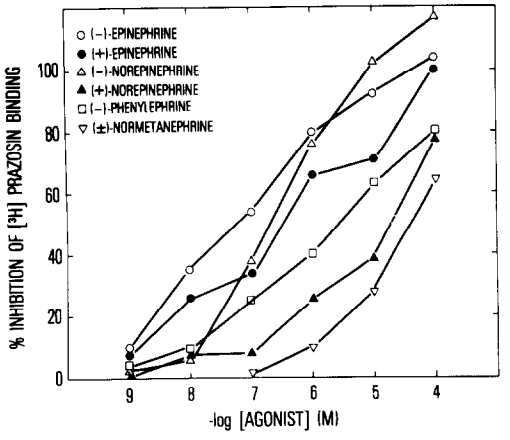

Fig. 6. Displacement of [^{3}H]prazosin binding from rat parotid membranes by adrenergic agonists. Parotid membranes were incubated at 20° for 30 min with 1 nM [^{3}H]prazosin and various concentrations of (−)-epinephrine (○), (+)-epinephrine (●), (−)norepinephrine (△), (+)-norepinephrine (▲), (−)-phenylephrine (□) and (±)-normetanephrine (▽). Maximum binding refers to the concentration of [^{3}H]prazosin specifically bound in the absence of any agonist. Each point is the mean of two or three experiments performed with triplicate determinations.

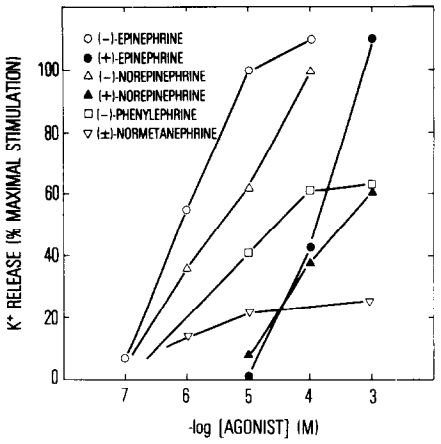

Fig. 7. Stimulation of K^+ release from parotid cell aggregates by adrenergic agonists. K^+ release, stimulated by various concentrations of (−)-epinephrine (○), (+)-epinephrine (●), (−)-norepinephrine (△), (+)-norepinephrine (▲), (−)-phenylephrine (□), and (±)-normetanephrine (▽), was determined as described in Materials and Methods. Maximum stimulation refers to K^+ release in the presence of 10 μM (−)-epinephrine (not significantly different from 100 μM). Each point is the mean of two to five experiments performed with quadruplicate determinations.

cell aggregates was rapid, reaching a maximum at 60 sec after the (−)-epinephrine addition, and was followed by a gradual re-accumulation (H. Ito, B. J. Baum, and G. S. Roth, unpublished data). The potency of a variety of adrenergic agonists in stimulating K^+ release from parotid cell aggregates was (−)-epinephrine > (−)-norepinephrine > (−)-phenylephrine ≫ (±)-normetanephrine (Fig. 7). The relative potency of a variety of adrenergic antagonists in inhibiting 10^{-5} M (−)-epinephrine-stimulated K^+ release was prazosin ≥ phenoxybenzamine ≫ yohimbine ≫ (±)-propranolol (Fig. 5). (−)-Epinephrine and (−)-norepinephrine were much more potent in stimulating K^+ release than were the (+)-isomers (Fig. 7).

Correlation between [^{3}H]prazosin binding to parotid membranes and K^+ release from parotid cell aggregates. The dose–response curves of a variety of adrenergic antagonists, agonists and the (±)-isomers in displacing [^{3}H]prazosin binding from parotid membranes correlate reasonably well with those in stimulating K^+ release from parotid cell aggregates (compare Figs. 5–7). Table 1 summarizes the K_D values of various adrenergic agents for displacing [^{3}H]prazosin binding to parotid membranes, the K_i values of antagonists for inhibiting (−)-

Table 1. Interaction of adrenergic agents with [^{3}H]prazosin binding sites in parotid membranes and with K^+ release from parotid cell aggregates*

	[^{3}H]Prazosin binding site	K^+ release	
	K_D (nM)	K_i (nM)	EC_{50} (nM)
Antagonists			
Prazosin	2	0.74	
Phenoxybenzamine†	6.7	1.1	
Yohimbine	1,300	300	
(±)-Propranolol	>38,000	>7,400	
Agonists			
(−)-Epinephrine	25		800
(−)-Norepinephrine	83		1,300
(−)-Phenylephrine	1,000		11,000
(±)-Normetanephrine	17,000		>1,000,000
(+)-Epinephrine	250		120,000
(+)-Norepinephrine	15,000		720,000

* The K_D, K_i and EC_{50} values were determined as described in the legend of Fig. 8. Results are the average of two to five experiments performed in triplicate or quadruplicate determinations.

† Since phenoxybenzamine is an irreversible antagonist, the values of K_D and K_i presented here are only apparent and used to compare that agent with the other adrenergic agents stated.

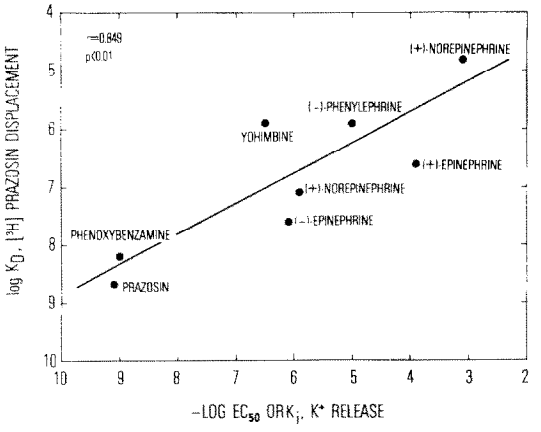

Fig. 8. Correlation between displacement of [^{3}H]prazosin binding from rat parotid membranes and inhibition or stimulation of K^+ release from parotid cell aggregates by adrenergic agents. The ability of adrenergic agents to displace [^{3}H]prazosin binding from membrane binding sites (K_D) and the ability to inhibit (−)-epinephrine-simulated K^+ release (K_i) were calculated from the dose–response curves of Figs. 5 and 6 according to the method of Cheng and Prusoff [19]. The EC_{50} value of K^+ release was determined for adrenergic agonists by replotting K^+ release dose–response curves of Fig. 7 on probit paper, and determining the concentration of agonist causing half-maximal release.

epinephrine-stimulated K^+ release, and the EC_{50} values of agonists for stimulating K^+ release from parotid cell aggregates. The correlation between the K_D and the K_i or the EC_{50} values is shown in Fig. 8. The ability of a number of adrenergic agents to displace [^{3}H]prazosin binding from parotid membranes was significantly correlated with the potency of those agents to stimulate or inhibit K^+ release from parotid cell aggregates ($r = 0.849$, $P < 0.01$).

DISCUSSION

Strittmatter *et al.* [9] have reported a good correlation of α-adrenergic antagonist ([^{3}H]DHE) binding to both parotid membranes or dispersed parotid cells with K^+ release from these cells. However, since [^{3}H]DHE can bind to both α_1- and α_2-adrenergic receptors (Fig. 1) in parotid glands, as well as in other tissues [5, 26–28], more detailed evaluation of the α-adrenergic receptor subtypes is required to understand their role in mediation of K^+ release from parotid cells.

There have been several proposed methods for measuring α_1- and α_2-adrenergic receptors separately. [^{3}H]WB4101 or [^{3}H]clonidine have been used as presumably α_1- and α_2-selective radioligands [4]. However, recent studies have questioned the specificity of these compounds [29, 30]. On the other hand, Hoffman *et al.* [5, 27] have analyzed the ratio of α_1- and α_2-adrenergic receptors by computing the ratio of the number of [^{3}H]DHE binding sites with a high K_D value for a selective α_1-antagonist, prazosin, and those with a high K_D value for a selective α_2-antagonist, yohimbine. Miach *et al.* [25] have also estimated α_1- and α_2-adrenergic receptors in brain tissue by assaying [^{3}H]DHE binding in the presence of yohimbine and prazosin respectively. An attempt to use this approach with parotid membranes was not entirely successful. The prazosin displacement curve resulted in a clear biphasic pattern (Fig. 1A) with parotid membranes. However, the yohimbine displacement curve to [^{3}H]DHE binding did not show a clear biphasic pattern (Fig. 1B) as was seen with the α-adrenergic receptors in brain tissue [25]. This result suggests a likely difficulty in characterizing α-adrenergic receptors in parotid membranes directly by assaying [^{3}H]DHE binding in the presence of yohimbine according to Miach *et al.* [25].

Therefore, we decided to evaluate α_1-adrenergic receptor populations by direct prazosin binding studies. The specific binding of [^{3}H]prazosin to rat parotid membranes is a rapid (Fig. 2), saturable (Fig. 4), reversible (Fig. 3), and stereospecific process (Fig. 6). The K_D value of 0.38 nM calculated from saturation studies (Fig. 4, A and B) is in good agreement with that of 0.48 nM calculated from kinetic studies (Fig. 2, A and B and Fig. 3, A and B). The maximum binding capacity of 380 fmoles/g tissue is also in good agreement with that of about 600 fmoles/g tissue calculated from [^{3}H]DHE binding studies according to the following formula: B_{max} of [^{3}H]DHE binding $\times \alpha_1/\alpha_1 + \alpha_2$.

Phenoxybenzamine, a selective α-adrenergic antagonist, was far more potent than (±)-propranolol, a selective β-adrenergic antagonist, in displacing [^{3}H]prazosin binding (Fig. 5, Table 1) and in inhibiting (−)-epinephrine-stimulated K^+ release (Fig. 5, Table 1). Furthermore, prazosin (K_D = 2 nM), a selective α_1-adrenergic antagonist, was 650-fold more potent than yohimbine (K_D = 1.3 μM), a selective α_2-adrenergic antagonist, in displacing [^{3}H]prazosin binding from parotid membranes. These agents displaced [^{3}H]prazosin binding in a monophasic pattern (Fig. 5). These results clearly demonstrated that [^{3}H]prazosin bound to only one class of sites, α_1-adrenergic receptors, in rat parotid membranes.

We next examined the effect of various agents on a physiological α-adrenergic response in rat parotid, K^+ release. The same order difference in potencies for inhibition of (−)-epinephrine-stimulated K^+ release from parotid cell aggregates, 400-fold, was found between prazosin ($K_i = 0.74$ nM) and yohimbine ($K_i = 0.3$ μM) (Fig. 5, Table 1) as was found for displacement of [^{3}H]prazosin binding, 650-fold (Fig. 5, Table 1). Moreover, phenoxybenzamine, reported as a relatively more selective antagonist for α_1-adrenergic receptors than for α_2-adrenergic receptors [3, 23], was almost equally potent as prazosin in competing with [^{3}H]prazosin binding (K_D = 6.7 nM while $K_D = 2.0$ nM for prazosin) (Fig. 5, Table 1) and in inhibiting (−)-epinephrine-stimulated K^+ release ($K_i = 1.1$ nM while $K_i = 0.74$ nM for prazosin) (Fig. 5, Table 1).

(−)-Phenylephrine is a more active adrenergic agonist on α_1-adrenergic receptors than (±)-normetanephrine [3, 31, 32]. As shown in Fig. 6 and Table 1, (−)-phenylephrine was substantially more efficient in inhibiting [^{3}H]prazosin binding (K_D = 1.0 μM) than (±)-normetanephrine (K_D = 17 μM), and was also more efficient in stimulating K^+ release

($EC_{50} = 11\ \mu M$ vs $EC_{50} > 1$ mM). In addition, (−)-norepinephrine ($K_D = 0.083\ \mu M$) was only 12-fold more potent than (−)-phenylephrine ($K_D = 1.0\ \mu M$) in displacing [^{3}H]prazosin binding from parotid membranes (Fig. 6, Table 1), and only 8.5-fold more potent ($EC_{50} = 1.3\ \mu M$ vs $EC_{50} = 11\ \mu M$) in stimulating K^+ release from parotid cell aggregates (Fig. 7, Table 1). The relative potencies of these two adrenergic agonists are consistent with those reported in α_1-adrenergic receptors (7-fold) but not those for α_2-adrenergic receptors (142-fold) [3].

Finally, the ability of a number of adrenergic agents to displace [^{3}H]prazosin binding from parotid membranes was correlated well with the potency of those agents in stimulating or inhibiting K^+ release from parotid cell aggregates (Fig. 8, Table 1).

The results presented here clearly demonstrate that [^{3}H]prazosin binding sites in parotid membranes are of α_1-adrenergic receptor character and that K^+ release from parotid cell aggregates is mediated via α_1-adrenergic receptors. Both the characteristics of [^{3}H]prazosin binding and catecholamine-stimulated K^+ release are consistent with this interpretation.

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