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Postsynaptic *Alpha*$_2$-Adrenoceptors Predominate over *Alpha*$_1$-Adrenoceptors in Canine Tracheal Smooth Muscle and Mediate Neuronal and Hormonal *Alpha*-Adrenergic Contraction

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SUMMARY

Alpha-adrenoceptor subtypes in canine tracheal smooth muscle were investigated by radioligand binding and by *in vitro* responses of muscle strips to electrical field stimulation and exogenous *alpha*-agonists. [^{3}H]Yohimbine identified a high density of *alpha*$_2$-receptors (51.4 ± 4.9 fmoles/mg of protein; n = 5) in tracheal smooth muscle membranes, whereas [^{3}H]prazosin revealed a low density of *alpha*$_1$-receptors (11.1 ± 2.9 fmoles/mg of protein; n = 5). In peripheral lung membranes, however, *alpha*$_1$-receptors predominated (46.8 ± 7.7 fmoles/mg of protein; n = 4) over *alpha*$_2$-receptors (4.1 ± 1.5 fmoles/mg of protein; n = 4). After pretreatment with atropine and propranolol and precontraction with serotonin or histamine, the contractile response of tracheal smooth muscle to electrical field stimulation was partially inhibited by 0.3 μM prazosin (16%), potently inhibited by 0.3 μM yohimbine (89%), and abolished by a combination of the two drugs. The response to neuronally released norepinephrine is therefore mediated predominantly by *alpha*$_2$-receptors. The rank order of potency for adrenergic agonists was clonidine > norepinephrine > phenylephrine in both competition studies with [^{3}H]yohimbine and in contraction studies, signifying a predominance of postsynaptic *alpha*$_2$-receptors. The contractile responses to exogenous norepinephrine, clonidine, and phenylephrine were only weakly inhibited by 0.3 μM prazosin but markedly inhibited by 0.3 μM yohimbine, with a K_b of 1.2 nM, which was similar to the K_d of [^{3}H]yohimbine binding to airway smooth muscle membranes (2.7 nM).

INTRODUCTION

Alpha-adrenoceptors which mediate contraction have been demonstrated in airway smooth muscle of several species, including dogs and humans (1–9). Several investigators have found *alpha*-agonist-mediated contraction to be present in canine tracheal smooth muscle *in vitro* or *in vivo* only when precontracted with histamine, serotonin, or potassium (4, 7–9), although others have reported *alpha*-adrenergic responses *in vivo* without prior muscle contraction in the presence of *beta*-blockade (5, 6).

Alpha-adrenoceptors may be classified into *alpha*$_1$- and *alpha*$_2$-subtypes based on relative potencies of a series of agonists and antagonists (10, 11). It was initially believed that *alpha*$_2$-receptors were localized postsynaptically and that *alpha*$_2$-receptors were presynaptic, inhibiting the release of norepinephrine from the nerve terminal (12), but it has recently been established that *alpha*$_2$-receptors may also be present postsynaptically in several tissues (13). It has been suggested that, while *alpha*$_1$-receptors are preferentially activated by norepinephrine released from sympathetic nerves, postsynaptic *alpha*$_2$-receptors may be localized extrasynaptically and preferentially regulated by circulating catecholamines (14). Both *alpha*$_1$- and *alpha*$_2$-receptors may exist in canine tracheal smooth muscle, as both prazosin (an *alpha*$_1$-antagonist) and yohimbine (an *alpha*$_2$-antagonist) may have inhibitory effects on *alpha*-agonist-induced contraction *in vivo* (15).

Recently, it has been possible to study *alpha*-receptor subtypes by direct radioligand binding assay, using [^{3}H]prazosin to bind to *alpha*$_1$-receptors and [^{3}H]yohimbine to *alpha*$_2$-receptors. We have investigated *alpha*-adrenergic receptors in canine tracheal smooth muscle using these radioligands. For comparison, we determined the pharmacological characteristics of the *alpha*-adrenergic response in isolated tracheal smooth muscle using both

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nerve stimulation and exogenous *alpha*-adrenergic agonists to activate the *alpha*-adrenoceptors.

METHODS

Membrane preparation. Tracheae were rapidly removed from mongrel dogs (15–25 kg) anesthetized with pentobarbitol sodium (30 mg/kg i.v.), and the posterior membrane was separated. The muscle, dissected free of connective tissue and epithelium, was finely minced in 10 volumes (w/v) of ice-cold incubation buffer (50 mM Tris HCl, pH 7.4) and homogenized in a Polytron tissue homogenizer (Brinkmann Instruments) at setting 8 for four 15-sec periods. The homogenate was filtered through two layers of cheesecloth, and the filtrate was centrifuged at 40° at 1200 × *g* for 10 min to remove unbroken cells and connective tissue. The supernatant was centrifuged at 30,000 × *g* for 15 min and the resulting pellet was washed and recentrifuged at the same speed. The final pellet was resuspended in buffer at a concentration of approximately 0.5 mg of protein per milliliter, and this particulate preparation was either used directly or stored at −70°. Storage for up to 2 months did not affect the binding characteristics. Protein was determined by the method of Bradford (16), using bovine serum albumin as the standard. Particulates were also prepared from peripheral lung which had been dissected free of cartilaginous airways and major blood vessels, using an identical procedure.

Binding assay. Particulates (approximately 100 μg of protein per assay) were incubated with radioligand ([^{3}H]prazosin or [^{3}H]yohimbine) in a final volume of 0.25 ml. Nonspecific binding was determined in separate incubations in the presence of 10 μM phentolamine. Each experimental point was determined in duplicate. Equilibrium incubations were carried out at 25° for 15 min with [^{3}H]prazosin, or for 30 min with [^{3}H]yohimbine. Incubations were terminated by dilution with 5 ml of ice-cold buffer and rapid filtration through Whatman GF/C filters, followed by two 5-ml washes. Filters were counted by liquid scintillation spectrometry at an efficiency of 50%.

Contraction studies. Each excised trachea was immediately immersed in oxygenated Krebs-Henseleit solution of the following composition: NaCl, 118 mM; KCl, 5.9 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; NaH_2PO_4, 1.2 mM; $NaHCO_3$, 25.5 m; glucose, 5.6 mM; penicillin-streptomycin, 100 units/m. The trachealis muscle was cleared of loose connective tissue and the epithelium was separated from the muscle, which was then cut into cross-sectional strips 2–3 mm wide. Six strips were mounted in glass chambers filled with 18 m of Krebs-Henseleit solution at 37° and constantly aerated with 94% O_2 and 6% CO_2. Isometric tension was measured by strain gauge (Grass FT .03 force-displacement transducer) and recorded continuously (Grass Model 7D polygraph). The tissue baths were fitted with platinum electrodes for electrical field stimulation using biphasic pulses (pulse duration, 0.5 msec; frequency, 12 Hz; supramaximal voltage for 20 sec). The strips were allowed to equilibrate for 1 hr while resting tension was adjusted to 10 g, which was found to be optimal for determining changes in tension. After equilibration, the strips were contracted by field stimulation, and only strips developing an increase in tension of greater than 10 g were included in the study. The response to field stimulation was determined every 6 min until stable. Cholinergic responses were then blocked by 1 μM atropine and *beta*-adrenergic responses by 1 μM propranolol. After 30 min, field stimulation gave no response, confirming cholinergic blockade. Either 0.3 μM serotonin or 30 μM histamine was added to all of the chambers to produce approximately 50% of the contraction produced by field stimulation in the absence of the blockers (the cholinergic response). Yohimbine (0.3 μM) was added to two chambers, prazosin (0.3 μM) to another two chambers, and the remaining two chambers served as controls. In some experiments, the effects of combining 0.3 μM yohimbine and 0.3 μM prazosin and the effects of 0.1 μM yohimbine were also investigated. After 30 min, tissues were stimulated electrically; cumulative dose-responses to (−)-norepinephrine (0.01–100 μM), clonidine (0.1–3000 μM), or (−)-phenylephrine (0.1–1000 μM) were then determined using a 2-min exposure time. In some experiments, cumulative dose-responses to *alpha*-agonists were performed in the absence of serotonin or histamine.

Drugs and chemicals. Drugs were obtained from the following sources: yohimbine hydrochloride, (−)-norepinephrine hydrochloride, (−)-phenylephrine hydrochloride, (±)-propranolol hydrochloride, (−)-isoproterenol, histamine diphosphate, serotonin creatinine sulfate, acetylcholine chloride (Sigma Chemical Company, St. Louis, Mo.); prazosin hydrochloride (Pfizer Laboratories, New York, N. Y.); clonidine hydrochloride (Boerhinger-Ingelheim, Ridgefield, Conn.); phentolamine mesylate (Ciba-Geigy, Summit, N. J.). [^{3}H]prazosin (specific activity, 20.2 Ci/mmole) was purchased from Amersham Corporation (Arlington Heights, Ill.) and [^{3}H]yohimbine (specific activity 82.6 Ci/mmole) from New England Nuclear Corporation (Boston, Mass.). All drugs were made up in distilled water immediately prior to use, and catecholamines were made up in 100 μM ascorbic acid.

Statistics. Results are reported as mean ± standard error; statistical significance was determined by the Mann-Whitney *U*-test.

RESULTS

[^{3}H]Yohimbine binding. Specific binding of [^{3}H]yohimbine to dog trachealis membranes comprised 80% of total binding and was saturable. Scatchard analysis showed a single class of high-affinity binding sites with a mean dissociation constant (K_d) of 2.7 ± 0.25 nM (n = 5) and maximal binding capacity (B_{max}) of 51.4 ± 4.9 fmoles/mg of protein (Fig. 1). Specific binding reached equilibrium by 20 min, was stable for 60 min, and was rapidly reversible on addition of phentolamine (Fig. 2). Analysis of the kinetics of binding gave a K_d of 2.0 ± 0.23 nM (n = 3), which was in good agreement with the K_d determined at equilibrium. Specific binding was completed by agonists in the rank order: Clonidine > (−)-epinephrine = (−)-norepinephrine > (−)-phenylephrine > (−)-isoproterenol, consistent with binding to *alpha*$_2$-receptors (Table 1). (−)-Norepinephrine was 200 times more potent than (+)-norepinephrine, confirming that binding was stereoselective. Antagonists competed for [^{3}H]yohimbine binding in the rank order: yohimbine > phentolamine > prazosin, again indicating that binding was to *alpha*$_2$-receptors. Neither (−)-propranolol nor atropine affected binding at a concentration of 10 μM. By contrast, there was very little specific binding of [^{3}H] yohimbine to peripheral lung membranes. Incubation of

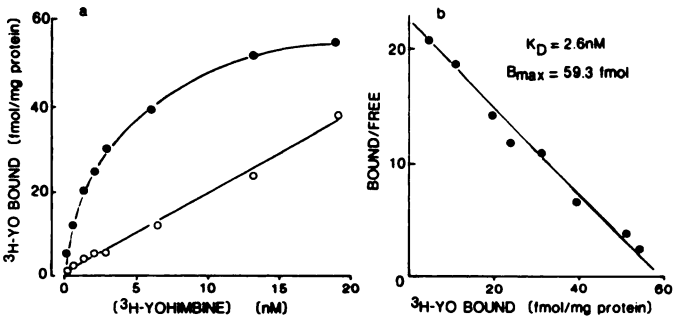

FIG. 1. *[^{3}H]Yohimbine binding to canine airway smooth muscle membranes*

a, Specific binding (●) of [^{3}H]yohimbine with increasing concentration. Nonspecific binding in the presence of 10 μM phentolamine is also shown (○). b, Scatchard analysis of specific [^{3}H]yohimbine binding showing a single class of binding site with a dissociation constant (K_d) of 2.6 nM, and a maximal concentration of binding sites (B_{max}) of 59.3 fmoles/mg of protein. The line was fitted by linear regression analysis (r = 0.98). The data shown are typical of five such experiments performed in duplicate, which gave a mean K_d of 2.7 ± 0.25 nM and mean B_{max} of 51.4 ± 4.9 fmoles/mg of protein.

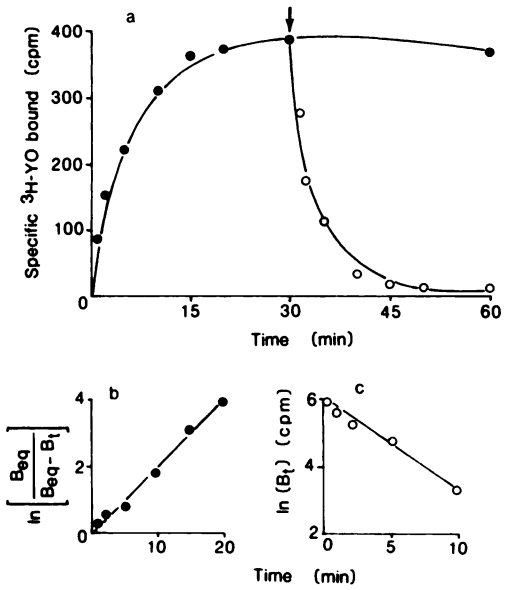

FIG. 2. *Kinetics of specific [^{3}H]yohimbine binding to canine airway smooth muscle membranes*

a, Time course of specific [^{3}H]yohimbine binding at 25° (●), at a ligand concentration of 1.8 nM. Phentolamine (10 μM) was added at the time indicated by the *arrow*, and the time course of dissociation was plotted (○). b, Pseudo-first order association plot of the data, in which B_{eq} is the amount bound at equilibrium and B_t is the amount bound at each time. The slope (r = 0.98) gave the observed association constant k_{obs} (0.199), giving a bimolecular rate constant association (k_1) of 0.11 min^{-1} nM^{-1}. c, First-order dissociation plot with the rate constant for dissociation (k_{-1}) given by the slope (0.25 min^{-1}). The kinetic dissociation constant (K_d), calculated from the ratio k_{-1}/k_1, was 2.3 nM. The data shown are typical of three such experiments, giving a mean kinetic K_d of 2.1 ± 0.3 nM.

particulates of peripheral lung with 10–12 nM [^{3}H]yohimbine, which should occupy >95% of *alpha*$_2$-adrenergic binding sites, resulted in specific binding of 4.1 ± 1.5 fmoles/mg of protein (n = 4) (Fig. 3).

TABLE 1

Inhibitory dissociation constants (K_i) of various drugs competing for specific [^{3}H]yohimbine binding to canine airway smooth muscle membranes

Each value is the mean of three experiments performed in duplicate. K_i was determined from the equation $K_i = IC_{50}/(1 + [L]/K_d)$, where IC_{50} is the molar concentration of drug causing 50% inhibition of specific binding, [L] is the concentration of [^{3}H]yohimbine used in the assay (2–3 nM), and K_d is the dissociation constant of [^{3}H]yohimbine from equilibrium binding experiments (2.7 nM).

Drug	K_i
	M
Agonists	
Clonidine	3.6×10^{-8}
(−)-Epinephrine	2.8×10^{-7}
(−)-Norepinephrine	6.8×10^{-7}
(+)-Norepinephrine	5.7×10^{-5}
(−)-Phenylephrine	6.9×10^{-5}
(−)-Isoproterenol	1.0×10^{-4}
Antagonists	
Yohimbine	5.7×10^{-9}
Phentolamine	1.4×10^{-8}
Prazosin	2.7×10^{-6}

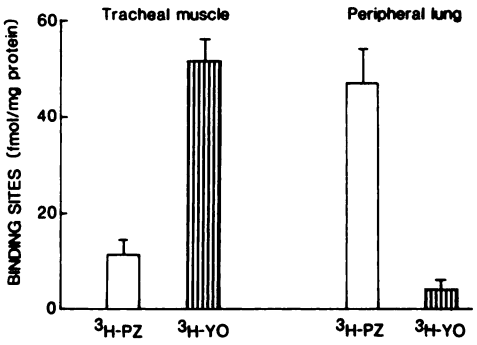

FIG. 3. *Concentration of [^{3}H]yohimbine and [^{3}H]prazosin binding sites in canine tracheal muscle and peripheral lung membranes*

The number of binding sites for [^{3}H]yohimbine in tracheal muscle and for [^{3}H]prazosin in lung were determined by Scatchard analysis. For [^{3}H]prazosin binding in tracheal muscle and for [^{3}H]yohimbine binding in lung, a single concentration of radioligand was used which should saturate >95% of receptors (for [^{3}H]prazosin, 5 nM; for [^{3}H]yohimbine, 12 nM). Values shown are mean ± standard error from four or five experiments.

[^{3}H]Prazosin binding. Because there was little specific binding of [^{3}H]prazosin to dog trachealis membranes, it was not possible with the amount of tissue available to construct a saturation curve or perform a Scatchard analysis. In peripheral lung membranes, specific binding of [^{3}H]prazosin comprised 70–80% of total binding and was saturable. Scatchard analysis gave a mean K_d of 0.61 ± 0.13 nM and B_{max} of 46.8 ± 7.7 fmoles/mg of protein (n = 4). Antagonists competed for [^{3}H]prazosin binding to canine lung particulates with the rank order: prazosin > phentolamine > yohimbine, confirming binding to *alpha*$_1$-receptors as previously described (17). If the K_d for [^{3}H]prazosin to tracheal smooth muscle membranes is assumed to be the same as to peripheral lung membranes, then a concentration of [^{3}H]prazosin of 5 nM should occupy > 95% of binding sites, so that an approximate B_{max} for [^{3}H]prazosin of binding can be determined (11.2 ± 2.9 fmoles/mg of protein; n = 5). When 10 nM [^{3}H]prazosin was used, there was no increase in specific binding (12.2 ± 4.2 fmoles/mg of protein; n = 3), indicating that specific binding sites were saturated.

Alpha-adrenergic contractile response. Addition of 1 μM atropine and 1 μM propranolol abolished the contractile response to electrical field stimulation. Serotonin (0.3 μM) caused baseline tension to increase by 16.7 ± 1.7 g (n = 30), and histamine (30 μM) caused tension to increase by 14.7 ± 1.0 g (n = 12). In control strips, electrical field stimulation then caused a contraction of 12.8 ± 1.0 g (n = 10), which amounted to 51.2 ± 4.9% of the response to field stimulation in the absence of atropine and propranolol (cholinergic response). In the presence of 0.3 μM prazosin, this contractile response to field stimulation was 10.8 ± 0.8 g (or 40.1 ± 3.1% of cholinergic response), which was slightly but significantly less than in control strips ($p < 0.05$). However, with 0.3 μM yohimbine, the response to field stimulation was only 1.4 ± 0.5 g (7.3 ± 2.3% of the cholinergic responses), which was very significantly less than the control response ($p < 0.001$). In the presence of both 0.3 μM yohimbine and 0.3 μM prazosin, the contraction and response to field stimulation were abolished (Fig. 4).

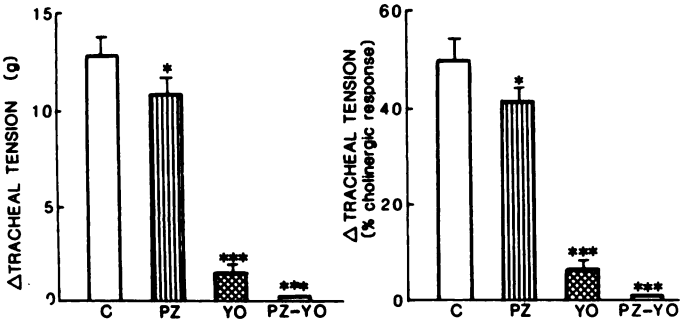

FIG. 4. *Effect of alpha-antagonists on electrical field stimulation-induced contraction in canine airway smooth muscle strips*

Left, increase in isometric tension in response to field stimulation (at 12 Hz for 20 sec) after pretreatment with 1 μM atropine, 1 μM propranolol, and 0.3 μM serotonin in control strips (*C*) and in the presence of 0.3 μ prazosin (*PZ*), 0.3 μM yohimbine (*YO*), and combined prazosin and yohimbine blockade (*PZ-YO*). Right, the same data plotted as a percentage of the contractile response to field stimulation in the absence of atropine and propranolol (cholinergic response). The data shown are mean ± standard error of 10 separate strips. Significance of difference from control: $*p < 0.05$; $***p < 0.001$.

In the absence of serotonin or histamine, there was no contractile response to maximal concentrations of norepinephrine, clonidine, or phenylephrine. In control strips, addition of norepinephrine in the presence of histamine and serotonin caused a dose-dependent increase in tension (maximum 17.4 ± 1.8 g; $n = 6$). The mean concentration of norepinephrine causing half-maximal contraction (EC_{50}) was 0.71 μM (Fig. 5). In the presence of prazosin, there was a parallel shift to the right of the dose-response curve, giving an EC_{50} of 1.8 μM. In the presence of 0.3 μM yohimbine, there was complete inhibition of contraction in response to norepinephrine and a progressive relaxation at higher concentrations, presumably due to the *beta*-adrenergic effect of norepinephrine's overcoming propranolol blockade.

A similar dose-dependent contractile response was also found to clonidine (EC_{50} = 0.63 μM) and phenylephrine (EC_{50} = 13 μM), giving a rank order of potency among *alpha*-agonists of clonidine > norepinephrine > phenylephrine. Prazosin had little inhibitory effect on the response to clonidine (EC_{50} = 2.0 μM), but had a more potent effect on the response to phenylephrine (EC_{50} = 40 μM). Yohimbine (0.3 μM) had a potent inhibitory effect on the dose-response to clonidine, causing a marked parallel shift (EC_{50} = 220 μM). The apparent dissociation constant (K_b) was determined as 1.2 nM from the relationship:

$$K_b = \frac{[I]}{(L'/L - 1)}$$

where $[I]$ is the concentration of antagonist, L' is the EC_{50} of the agonist in the presence of antagonist, and L is the EC_{50} of the agonist alone. This relationship assumes that a Schild plot would have a slope of unity, and that this was so is supported by finding that a lower concentration of yohimbine (0.1 μM), which caused less shift in the dose-response curve to clonidine, gave the same K_b (1.3 nM). The K_b determined from contractile studies was very similar to the K_d of [^{3}H]yohimbine determined from the binding studies (2.7 nM).

DISCUSSION

Direct binding studies showed that both *alpha*$_1$- and *alpha*$_2$-adrenoceptors are present in canine tracheal smooth muscle and that *alpha*$_2$-adrenoceptors predominate over *alpha*$_1$-receptors by a ratio of approximately 5:1. Binding of [^{3}H]yohimbine to smooth muscle particulates was saturable, reversible, and stereoselective and had the specificity expected of interactions with *alpha*$_2$-adrenoceptors. The affinity of [^{3}H]yohimbine to airway smooth muscle was similar to that determined in canine arteries (18) and human platelets (19). The density of *alpha*$_2$-receptors in tracheal smooth muscle greatly exceeded that in peripheral lung (by an approximate ratio

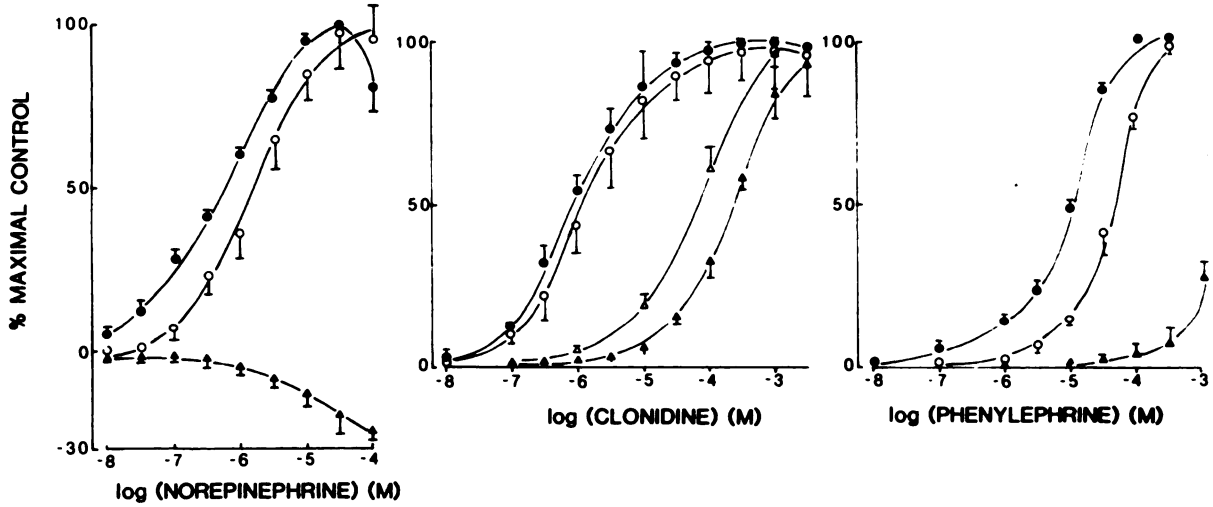

FIG. 5. *Contraction of canine trachealis strips by exogenous alpha-adrenergic agonists after precontraction with serotonin*

All strips were pretreated with 1μM atropine, 1 μM propranolol, and 0.3 μM serotonin. Responses are expressed as percentage of the maximal response in control strips. Left, response to norepinephrine in control stripes (●) and in strips after pretreatment with 0.3 μM prazosin (○) and 0.3 μM yohimbine (▲). Middle, response to clonidine in control strips (●) and after pretreatment with 0.3 μM prazosin (○), 0.1 μM yohimbine (△), or 0.3 μM yohimbine (▲). Right, response to phenylephrine in control strips (●) and after pretreatment with 0.3 μM prazosin (○) and 0.3 μM yohimbine (▲). Each point represents the mean ± standard error of three to six separate strips.

of 12:1). [^{3}H]Prazosin binding to canine lung membranes was saturable, with an affinity similar to that determined in guinea pig and human lung (17, 20) and with a specificity characteristic of binding to *alpha*$_1$-receptors. By contrast to the distribution of *alpha*$_2$-receptors, the density of *alpha*$_1$-receptors in peripheral lung was greater than that in tracheal smooth muscle by an approximate ratio of 4:1.

Our studies on the specificity of *alpha*-adrenoceptor-mediated contraction of canine airway smooth muscle confirmed that, although both *alpha*-receptor subtypes were present, contraction was mediated predominantly by *alpha*$_2$-receptors, whether nerve stimulation or exogenous norepinephrine were used to activate *alpha*-receptors. Electrical field stimulation at 12 Hz in the presence of atropine and propranolol caused no significant contraction unless the muscle was precontracted with histamine or serotonin. This is in agreement with previous reports that *alpha*-adrenergic contractile responses are seen in canine tracheal smooth muscle only after precontraction with histamine, serotonin, or potassium chloride (4, 7–9). Field stimulation with the stimulus parameters described alters smooth muscle tone by releasing neurotransmitter from postganglionic nerves in airway smooth muscle rather than by a direct effect on smooth muscle cells (21). Since cholinergic and *beta*-adrenergic receptors were blocked, the contraction observed was presumably due to norepinephrine release and activation of postsynaptic *alpha*-receptors. The contraction was only partially inhibited (16%) by 0.3 μM prazosin, but almost abolished (89%) by yohimbine at the same concentration, indicating that the *alpha*-adrenergic response to nerve stimulation is mediated predominantly by *alpha*$_2$-receptors. Since the response was significantly reduced by prazosin, and the residual contraction after yohimbine abolished after addition of prazosin, it is likely that *alpha*$_1$-receptors mediate a small proportion of the *alpha*-adrenergic response to nerve stimulation.

In the presence of atropine and propranolol, and when the muscle was precontracted by serotonin and histamine, exogenous norepinephrine, which is an equally effective agonist for *alpha*$_1$- and *alpha*$_2$-receptors, caused a dose-dependent contraction. The dose-response to norepinephrine was only slightly shifted to the right in the presence of 0.3 μM prazosin, indicating that *alpha*$_1$-receptors mediate only a small component of the *alpha*-adrenergic response to exogenous agonists. However, with a 0.3 μM yohimbine there was a complete inhibition of contractile response to norepinephrine, which produced a dose-dependent relaxation as higher concentrations overcame *beta*-adrenoceptor blockade by propranolol. These results indicate that the *alpha*-adrenoceptor-induced contraction in response to exogenous norepinephrine, as with neuronally released norepinephrine, is mediated predominantly by *alpha*$_2$-adrenoceptors. Further evidence for the predominance of *alpha*$_2$receptors over *alpha*$_1$-receptors in mediating the contractile response is provided by the effects of selective *alpha*-agonists. The *alpha*$_2$-selective agonist clonidine caused a contractile response similar to that induced by norepinephrine, although there was only minimal inhibition by 0.3 μM prazosin. Both 0.1 and 0.3 μM yohimbine, however, produced marked inhibition of this response. The dissociation constant for yohimbine derived from its inhibitory effect on clonidine-induced contraction (1.2 nM) was in good agreement with that determined for [^{3}H]yohimbine binding to smooth muscle membranes (2.7 nM). The *alpha*$_1$-selective agonist phenylephrine was less potent than norepinephrine and clonidine in causing a contractile response, giving a rank order of potencies for *alpha*-agonists of clonidine > norepinephrine > phenylephrine, which is similar to the rank order of these agonists for inhibition of specific [^{3}H]yohimbine binding to smooth muscle membranes. This strongly suggests that the postsynaptic *alpha*-receptor mediating the *alpha*-adrenergic contractile response in dog tracheal smooth muscle is predominantly of the *alpha*$_2$-subtype. This is in good agreement with the binding studies showing that the majority of *alpha*-receptors in tracheal smooth muscle belong to the *alpha*$_2$-subtype. That *alpha*$_1$-receptors may also contribute to the contractile response is suggested by the finding that prazosin causes more inhibition of contraction induced by *alpha*$_1$-selective phenylephrine than to *alpha*$_2$-selective clonidine.

When *alpha*-adrenoceptors were initially subclassified, it was believed that *alpha*$_2$-receptors were localized presynaptically on nerve terminals, whereas *alpha*$_1$-receptors were localized to postsynaptic membranes (12). There is now increasing evidence that postsynaptic *alpha*$_2$-receptors, in addition to *alpha*$_1$-receptors, may mediate contraction in smooth muscle of several tissues, including arteries, veins, vas deferens, and anococcygeus (13, 22, 23). Our finding that exogenous *alpha*-adrenergic agonists caused predominantly *alpha*$_2$-mediated contraction in airway smooth muscle suggests that the *alpha*$_2$-receptors in airway smooth muscle are located postsynaptically. Furthermore, the high density of *alpha*$_2$-receptors determined by direct binding assay strongly suggests that these *alpha*$_2$-receptors are localized on smooth muscle membranes, since the proportion of the particulate preparation derived from sympathetic nerves must be very small in proportion to that derived from smooth muscle cells. Airway smooth muscle appears to be unusual in that, in contrast to vascular smooth muscle (18), *alpha*$_2$-receptors predominate over *alpha*$_1$-receptors. It is possible that this could be related to the paucity of sympathetic innervation in smooth muscle of the airways, in comparison to that in blood vessels (24). The responses to both sympathetic nerve stimulation and to exogenous agonists are equally effectively inhibited by yohimbine. This provides no support for the hypothesis that neuronally released norepinephrine selectively activates postsynaptic *alpha*$_1$-receptors, whereas exogenous norepinephrine (and circulating catecholamines) preferentially activates "extrajunctional" postsynaptic *alpha*$_2$-receptors, as suggested for vascular smooth muscle (14).

The importance of smooth muscle *alpha*-adrenoceptors in the control of airway smooth muscle tone, and particularly in airway disease, remains uncertain. The predominance of *alpha*$_2$-receptors over *alpha*$_1$-receptors, if also found in human airway smooth muscle, may explain why the nonselective antagonist phentolamine is more potent in producing bronchodilation and preventing bronchoconstriction in human asthmatic subjects (25–27) than the selective *alpha*$_1$-antagonist prazosin (28, 29). Although *alpha*$_2$-receptors predominate in tracheal

smooth muscle, it is possible that $alpha_1$-receptors may be more important in smaller airways. Indeed, the finding that the density of $alpha_1$-receptors in peripheral lung was very much greater than that of $alpha_2$-receptors supports this idea. By using [^{3}H]prazosin to localize $alpha_1$-receptors by light microscopic autoradiography (30), we have recently found a high density of $alpha_1$-receptors in smooth muscle of small airways in contrast to a low density in large airway smooth muscle, suggesting that there may be a difference between small and large airways (31). The physiological and pathophysiological significance of the coexistence of postsynaptic $alpha_1$- and $alpha_2$-adrenoceptors in airway smooth muscle requires further study, as it is possible that $alpha_1$- and $alpha_2$-receptors may be differentially regulated.

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