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Role of α_1-adrenoceptors and 5-HT_2 receptors in serotonin-induced contraction of rat prostate: autoradiographical and functional studies

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Abstract

Urinary obstruction from benign prostatic hyperplasia is a common clinical problem possibly associated with excessive prostatic constriction around the urethra. These studies compared adrenergic and serotonergic functional activity to specific α_1 and serotonin (5-hydroxytryptamine; 5-HT) binding sites in the rat prostate. Isolated, left ventral lobes of the rat prostate were removed and examined for *in vitro* contraction. Norepinephrine-induced contraction of the rat prostate was competitively blocked by prazosin with an apparent antagonist dissociation constant (pK_B) of 8.13. 5-HT also contracted the rat prostate. However, in the presence of prazosin, maximum 5-HT contraction was reduced by half suggesting that high concentrations of 5-HT can activate α_1 receptors in the prostate. The concentration-response curve to 5-HT in the presence of 1 μM prazosin was competitively inhibited by the 5-HT_2 receptor antagonist LY53857 (6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid 2-hydroxyl-1-methylpropylester (*Z*)-2-butenedioate (1:1)) ($pK_B = 9.02$). Autoradiographic studies with [^{125}I]LSD (2-iodo-lysergic acid diethylamide) documented the presence of 5-HT_2 receptors since significant displacement of the radioligand occurred with 5-HT and LY53857, but not with prazosin. The α_1-adrenoceptor ligand [^{125}I]HEAT ([β-(4-hydroxy-3-iodophenyl)ethyl-aminomethyl]-tetralone) confirmed the presence of α_1-adrenoceptors in the rat prostate since significant displacement of the radioligand occurred with prazosin, but not 5-HT or LY53857. The inability of prazosin to displace [^{125}I]LSD and the inability of 5-HT to displace [^{125}I]HEAT suggest that 5-HT cannot directly interact with α_1-adrenoceptors in the prostate. Thus activation of both α_1-adrenoceptors and 5-HT_2 receptors resulted in contraction of the rat prostate, and 5-HT-induced contraction was mediated by activation of 5-HT_2 receptors and the indirect activation of α_1-adrenoceptors.

Keywords: 5-HT_2 receptor; α-Adrenoceptor; Prostate, rat; Smooth muscle; Autoradiography

1. Introduction

Prostatic bladder obstruction due to benign prostatic hyperplasia is a common urological presentation in men over the age of 40. Until recently, surgical intervention was the only acceptable method of relief for these patients (Horby-Petersen et al., 1985). In 1991, it was estimated that 29% of men over the age of 40 will undergo surgical intervention due to benign prostatic hyperplasia (James et al., 1989). Thus there is great interest in the development of pharmacological agents for the non-surgical management of bladder outlet obstruction and benign prostatic hyperplasia.

In humans, α-adrenoceptor antagonists decrease pressure in the urethra and improve bladder outlet obstruction (Lepor et al., 1988) suggesting an important contribution of α-adrenoceptor activation to the symptoms of benign prostatic hyperplasia. A potential role for 5-HT (5-hydroxytryptamine, serotonin) was suggested by the studies of Horby-Petersen et al. (1985) who showed that ketanserin increased the maximum and mean urine flow rates in patients with benign prostatic hyperplasia. However, these studies did not discern whether the beneficial effect of ketanserin was due to its α-adrenoceptor or 5-HT receptor blocking effects or both. The prostate contains relatively high concentrations of 5-HT (Di Sant'Agnese et al., 1987;

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Hanyu et al., 1987; Crowe et al., 1991; Chapple et al., 1991). Neuroendocrine cells in the human prostate have been demonstrated to contain and release 5-HT among other paracrine acting substances (Falkmer et al., 1990). These neuroendocrine cells have been suggested to play a role in growth and differentiation of the prostate (Di Sant'Agnese, 1992). Whether these cells contribute to prostatic growth in benign prostatic hyperplasia is unknown; however, significant increases in neuroendocrine cell numbers have been demonstrated in prostatic adenocarcinoma (Di Sant'Agnese, 1992). Thus, in certain conditions, locally released 5-HT could play a role in prostatic functions. The presence of α_1-adrenoceptor and 5-HT receptors has been demonstrated in non-human mammalian prostatic tissues (Hedlund et al., 1985; Cohen and Drey, 1989). However, 5-HT receptors mediating contractility have not been extensively studied with regard to function or characterization raising the need to characterize such responses in more detail for prostatic smooth muscle.

Although the rat prostate contains significantly less smooth muscle than the human prostate, rat prostate resembles human tissue in that it contracts to α-adrenoceptor agonists and to 5-HT and the receptors mediating 5-HT-induced contraction are not well characterized (Cohen and Drey, 1989). 5-HT receptor characterization studies in other tissues have demonstrated that many rat 5-HT receptors have a similar pharmacological profile to the human receptor counterparts (Hen, 1992; Hartig et al., 1992). The present studies were designed (1) to examine the contribution of α-adrenoceptor vs. 5-HT receptor interactions to 5-HT-induced contraction in the isolated rat prostate, (2) to compare, using autoradiography, the relative density of specific α_1-adrenoceptor and 5-HT binding sites in the rat prostate, and (3) to characterize pharmacologically the contractile 5-HT receptors via the use of selective receptor antagonists.

2. Materials and methods

Sexually mature male Wistar-Kyoto rats (250–350 g) obtained from Harlan, (Indianapolis, IN, USA) were killed by rapid cervical dislocation. The prostate was exposed with a midline ventral incision and retraction of the vas differentia, bladder and associated tissues. The left ventral lobe was cleaned of the prostatic capsule according to a method previously described (Steidle et al., 1989) and gently placed in Krebs' bicarbonate buffer.

2.1. Functional studies

The lobes of the prostate were anchored in tissue baths with mercerized cotton thread with the longitudinal axis placed such that longitudinal contractions would draw vertically on the transducers. Tissue baths contained modified Krebs' bicarbonate buffer of the following composition (mM): NaCl 118.2; KCl 4.6; $CaCl_2$ 1.6; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 10.0; and $NaHCO_3$ 24.8, oxygenated with 95% O_2/5% CO_2 and maintained at 37°C. Resting force was set at 1 g as optimized previously (Cohen and Drey, 1989). Contractions were recorded with Statham UC3 transducers connected to a Beckman dynograph. After an equilibration period of at least 1 h, all tissues were challenged with 67 mM KCl which produced a contraction used to normalize subsequent responses. After maximum contraction was achieved (5–10 min) KCl was removed and tissues were allowed to return to baseline before challenge with other compounds. Cumulative concentration-response curves to 5-HT and norepinephrine in the presence (1 h) or absence of antagonists were generated.

The apparent dissociation constants (pK_B) were determined as previously described (Killam and Cohen, 1991) from EC_{50} values derived from fitting the hyperbolic agonist dose-response curve using a PC-based non-linear regression analysis package PCNONLIN (Statistical Consultants, Edgewood, KY, USA).

2.2. Autoradiographical studies

2.2.1. Tissue preparation

Prostate tissue was dissected in a procedure identical to that used for functional studies, cleaned of the prostatic capsule, and placed in modified Krebs' buffer. The left, ventral prostate lobes were oriented in plastic cryomolds for sectioning. The cryomolds containing tissue were filled with OCT embedding compound (OCT) (Miles Laboratories) and frozen in isopentane cooled to near the solidifying temperature for isopentane by immersion of the isopentane container in liquid nitrogen. The frozen OCT blocks were kept at −70°C until sectioning. Frozen prostate lobes were serially cross-sectioned rostral to caudal using a Cryocut microtome/freezer at −18°C at a thickness setting of 8 μm. Sections were thaw mounted to glass slides precoated with a subbing mixture of chrome alum and gelatin. Slides were refrozen and stored at −70°C until the binding studies. Sections from each animal's prostate were stained and studied together such that variations in size occurred as serial sectioning proceeded through the prostate lobe.

2.2.2. Autoradiography binding

Optimal binding conditions for [^{125}I]LSD (2-iodolysergic acid diethylamide) for the rat prostate were determined to be as follows: [^{125}I]LSD at 175 pM in 50 mM Tris-HCl buffer, pH 7.4; 4 h of incubation at room temperature followed by two 10 min washes in Tris

buffer at 4°C. Incubation was performed in covered containers to reduce light-induced deterioration of the ligand. Binding containers held either [^{125}I]LSD alone or [^{125}I]LSD plus a displacing non-radioactive ligand: 1 μM prazosin, 10 μM 5-HT, or 1 μM LY53857 (6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid 2-hydroxyl-1-methylpropyl ester (*Z*)-2-butenedioate). After the washes, a brief dip in ice-cold deionized water was followed by drying with a stream of cold dry air as outlined by Kuhar and Unnerstall (1990). Dried slides were placed in slide boxes, containing desiccant, overnight. Slides were then mounted on cardboard and placed in an x-ray cassette containing a sheet of β-Max hyper film. Exposure time for the [^{125}I]LSD autoradiograms was 20 days at 4°C. The β-Max hyper film was developed according to the manufacturer's suggestions using Kodak D-19 developer.

For the [^{125}I]HEAT ([β-(4-hydroxy-3-iodophenyl) ethyl-amino methyl]-tetralone) binding studies, the slide-mounted tissue slices were incubated with 50 pM [^{125}I]HEAT in 50 mM Tris-HCl, pH 7.4, containing 1 mM EDTA for 3 h at room temperature. Binding containers were prepared similarly to the LSD experiments with either [^{125}I]HEAT alone or the radioligand plus prazosin (1 μM), 5-HT (10 and 100 μM) or LY53857 (1 μM). Incubation was followed by three 10 min washes in 4°C binding buffer (no radioligand), dipped in 4°C deionized H_2O and dried as described for the [^{125}I]LSD studies. The slides were placed in cassettes with film as described above. The β-Max hyper film exposure time for the [^{125}I]HEAT autoradiograms was 4 days at 4°C.

To determine the cellular location of the binding sites, parallel binding studies with both [^{125}I]LSD and [^{125}I]HEAT were performed on serial prostate sections which were treated and dried identically to those sections apposed to the β-Max film with the exception that Kodak NTB-3 emulsion coverslips were mounted to the slides. The emulsion coated coverslips were mounted, developed, fixed and visualized as previously described (Killam and Cohen, 1992).

2.2.3. Determination of binding densities

Optical densities of the black and white images generated on the β-Max hyper film were quantified using a video-based image analysis system, MCID (Research Imaging, St. Catherine's, Ontario, Canada). Standard density images were generated on each film using [^{125}I]-microscales (from Amersham, Arlington Heights, IL, USA). The standard densities were quantified with the imaging system and converted to fmol/mg protein densities by the software using the equation of Nazarali et al. (1989). The densities of the prostate tissue images were then directly read by the imaging system from chosen areas. For each prostate image scanned in this way, 4–6 separate, randomly chosen areas were recorded. These values were used to determine statistical differences between the various treatment groups. Color-converted film images were photographed from the video screen using a Nikon 35 mm camera outfitted with a hood for photographing computer monitor images.

2.3. Statistics

To determine statistical differences between non-specific ligand treatment groups, all readings for all treatments were compared using analysis of variance (ANOVA), followed by Newman-Keuls' post-hoc test. Significance was assumed when $P < 0.05$.

2.4. Chemicals

[^{125}I]HEAT (specific activity 2200 Ci/mmol) and [^{125}I]LSD (specific activity 2200 Ci/mmol) were purchased from New England Nuclear division of DuPont, Boston, MA, USA. Tris buffer crystals, EDTA, 5-HT, and prazosin were purchased from Sigma Chemical Company, St. Louis, MO, USA. LY53857 was synthesized at Eli Lilly and Company, Indianapolis, IN, USA. β-Max hyper film was purchased from Amersham, Arlington Heights, IL, USA. Kodak chemicals were obtained from standard suppliers.

3. Results

3.1. Functional studies

Norepinephrine-induced contractions of the rat prostate were competitively blocked by prazosin (Fig. 1). The negative logarithm of the apparent antagonist

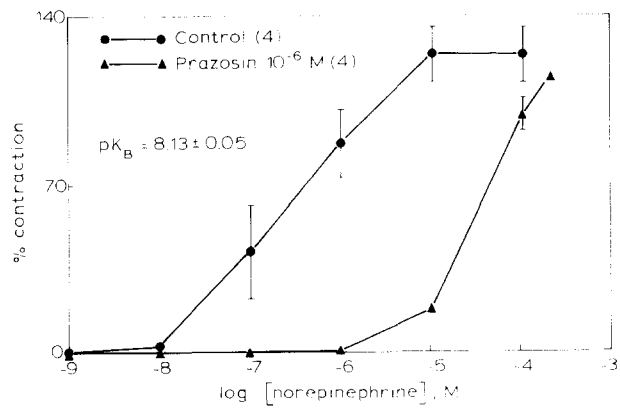

Fig. 1. The effect of prazosin (1 μM) on norepinephrine-induced contractions of the left ventral lobe of the rat prostate. Values represent mean ± standard error of the mean, expressed as percentage of contraction induced by 67 mM KCl. Number in parentheses represents the number of animals.

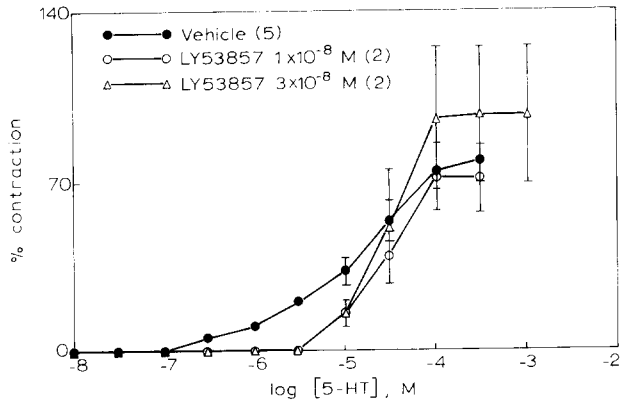

Fig. 2. The effect of two concentrations of LY53857 on 5-HT-induced contractions in the left ventral lobe of the rat prostate. Values represent mean ± standard error of the mean, expressed as percentage of contraction induced by 67 mM KCl. Number in parentheses represents the number of animals.

dissociation constant (pK_B) for prazosin against norepinephrine-induced contractions was 8.13 ± 0.05 (n = 4) suggesting that α_1-adrenoceptors were the predominant receptor responsible for contraction to norepinephrine.

5-HT also contracted the rat prostate; however, initial attempts to block the contractions with the 5-HT_2 receptor antagonist LY53857 produced complex curves (Fig. 2) with an apparent inhibition of contraction only to low concentrations ($< 10^{-5}$ M) of 5-HT. When 5-HT concentration-response curves were performed in the presence of prazosin (1 μM), maximum 5-HT contractile force was reduced by half suggesting that at higher concentrations of 5-HT ($> 10^{-5}$ M), 5-HT activated α_1-adrenoceptors in this tissue (Fig. 3).

When the studies with LY53857 were repeated in the presence of prazosin (1 μM) to minimize α_1-adrenoceptor interactions, LY53857 produced a parallel

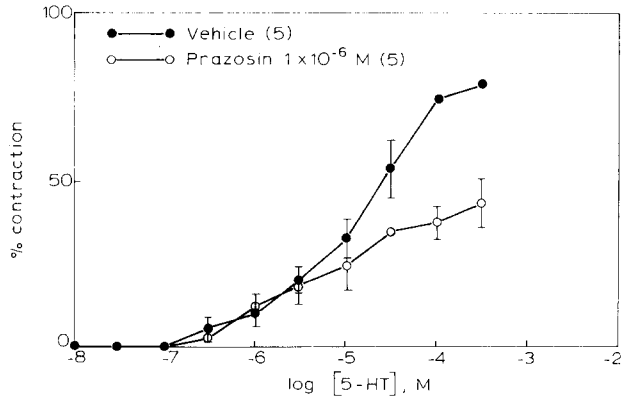

Fig. 3. The effect of prazosin (1 μM) on 5-HT-induced contractions of the left ventral lobe of the rat prostate. Values represent mean ± standard error of the mean, expressed as percentage of contraction induced by 67 mM KCl. Number in parentheses represents the number of animals.

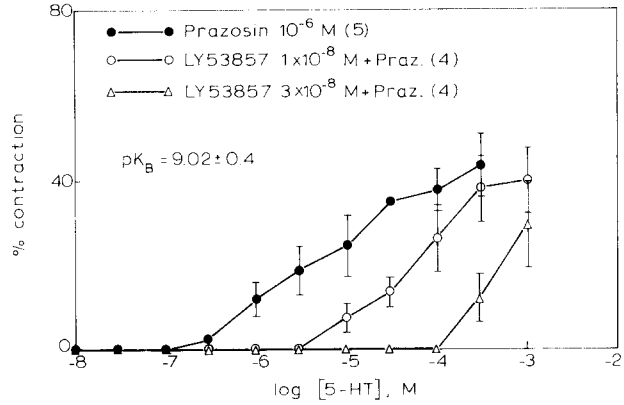

Fig. 4. The effect of two concentrations of LY53857 in the presence of 1 μM prazosin on 5-HT-induced contractions in the left ventral lobe of the rat prostate. Values represent mean ± standard error of the mean, expressed as percentage of contraction induced by 67 mM KCl. Number in parentheses represents the number of animals. pK_B value calculated from the concentration-response curve in the presence of 1×10^{-8} M LY53857.

dextral shift of the 5-HT concentration-response curve (Fig. 4). The negative logarithm of the antagonist dissociation constant derived for LY53857 under these conditions was 9.02 ± 0.4 (n = 4). Propranolol (10^{-6} M) had no effect on the norepinephrine- or 5-HT-induced contractions (data not shown).

3.2. Autoradiography

The displacement of the 5-HT receptor ligand [^{125}I]LSD by 5-HT and LY53857 revealed a small population of specific serotonergic binding sites. Fig. 5 presents representative serial autoradiograms from one prostate lobe. Table 1 represents the combined results of 3 experiments with tissue from 3 separate animals. Binding site densities for [^{125}I]LSD in the presence of 5-HT (1 μM) and LY53857 (1 μM) were significantly lower than the total binding sites in the absence of non-radioactive ligands ($P < 0.05$, ANOVA followed by Newman-Keuls' post-hoc test) (Table 1, Fig. 5). Incubation with prazosin (1 μM) did not significantly affect [^{125}I]LSD binding. Thus, [^{125}I]LSD appears to be binding to 5-HT_2 receptor sites and not to α_1-adrenoceptors under the conditions of this study.

Binding studies with the α-adrenoceptor ligand [^{125}I]HEAT were designed to identify prostatic α_1-adrenoceptors and to determine the extent to which 5-HT could bind to α_1-adrenoceptors in an effort to understand the effect of α_1-adrenoceptor antagonists to inhibit 5-HT-induced contraction in the rat prostate. Binding densities determined from these studies are shown in Table 2. Fig. 6 presents serial autoradiograms from one prostate lobe. Table 2 represents the combined results of 3 experiments with tissues from 3 separate animals. The α_1-adrenoceptor antagonist pra-

Table 1
Rat prostate [^{125}I]LSD binding sites (fmol/mg protein)

	Total	5-HT	LY53857	Prazosin
Mean	2.46	1.34 [a]	1.41 [a]	1.97
S.E.M.	0.17	0.12	0.13	0.21

$n = 3$(3 animals, 3 binding studies).
[a] Significantly different from total binding ($P < 0.05$).

Table 2
Rat prostate [^{125}I]HEAT binding sites (fmole/mg protein)

	Total	Prazosin	LY53857	5-HT
Mean	10.28	7.35 [a]	12.46	10.37
S.E.M.	0.60	0.47	0.83	1.46

$n = 3$ (3 animals, 3 binding studies).
[a] Significantly different from total binding ($P < 0.05$).

zosin (1 μM) greatly reduced [^{125}I]HEAT binding (significantly different from total binding, $P < 0.05$). Neither LY53857 nor 5-HT significantly displaced [^{125}I]HEAT from its binding sites (Table 2, Fig. 6). Thus, although the α_1-adrenoceptor antagonist prazosin inhibited the contractile response to high serotonin concentrations (Fig. 3), 5-HT did not measurably bind to [^{125}I]HEAT binding sites. Further, LY53857, a pan 5-HT$_2$ receptor antagonist, also did not bind to [^{125}I]HEAT sites confirming LY53857's specificity for 5-HT rather than α_1-adrenoceptors.

Under light microscope visualization, the emulsion-coated coverslip-mounted prostate sections revealed radioligand displacement profiles identical to the hyper film studies (data not shown). The specific binding could be confidently localized to the tissue. However, while it appeared that most of both the [^{125}I]HEAT and [^{125}I]LSD specific binding was localized to the stromal epithelium, the distance limitation of gamma particle scatter, and the very thin smooth muscle layers in the rat prostate did not permit specific determination of binding to the smooth muscle.

4. Discussion

Clinical studies using ketanserin in patients with bladder obstruction from benign prostatic hyperplasia showed that the urine flow was improved with ketanserin treatment (Horby-Petersen et al., 1985). Questions arose concerning whether the effect of ketanserin was due to α-adrenoceptor and/or 5-HT receptor antagonism of prostate contraction. Additionally, a significant increase in 5-HT containing and secreting neuroendocrine cells has been demonstrated in adenocarcinoma of the human prostate (Di Sant'Agnese, 1992). Release of 5-HT from these cells may contribute to secretory, contractile or proliferative functions of the prostate cell types. While the rat prostate has not been accepted as a model of human benign prostatic hyperplasia, rats have been used as models of chronic bacterial prostatitis and prostatic adenocarcinoma (Nickel et al., 1990; Omarbasha et al., 1989). Although rat prostate glands contain less smooth muscle than human prostate (Lepor et al., 1992), both tissues contract to adrenoceptor agonists and 5-HT (Hedlund et al., 1985; Cohen and Drey, 1989). We undertook these studies in the isolated rat prostate to determine the relative density of α-adrenoceptors and 5-HT receptors in rat prostate and the role of each of these receptors in the contractile response to 5-HT.

Norepinephrine is present in prostatic tissue (Baumgarten et al., 1968) and norepinephrine can contract the rat prostate (Cohen and Drey, 1989; this paper). These data in rat prostate are consistent with previous studies (Hedlund et al., 1985) that demonstrated the ability of human prostate to contract to the α_1-adrenoceptor agonist phenylephrine, and radiographical studies that documented the presence of α_1-adrenoceptors in human prostatic smooth muscle (James et al., 1989). Similarly, in the rat prostate, norepinephrine-induced contraction was markedly antagonized by the α_1-adrenoceptor antagonist, prazosin (Cohen and Drey, 1989).

The presence of α_1-adrenoceptors in the rat prostate was also confirmed with autoradiographical studies. Using the [^{125}I]HEAT, a ligand with high affinity for α_1-adrenoceptors (Glossman et al., 1991; Engel and Hoyer, 1981; Killam and Cohen, 1992), we have documented the presence of receptor binding sites in the rat prostate with α_1-adrenoceptor characteristics. These studies reinforce the previous suggestion that [^{125}I]HEAT is a useful ligand for autoradiographical detection of α_1-adrenoceptors in smooth muscle (Killam and Cohen, 1992), and document that the autoradiographical conditions used in the present study will permit detection of α_1-adrenoceptor binding.

As with norepinephrine, immunohistochemical or HPLC studies in human (Crowe et al., 1991; Di Sant'Agnese, 1985) and guinea pig (Davis, 1987) prostate have demonstrated the presence of 5-HT. To characterize the receptors mediating the contractile response to 5-HT in the rat prostate, we used LY53857 which, unlike ketanserin, is a pan 5-HT$_2$ receptor antagonist with minimal α_1-adrenoceptor affinity. The minimal α_1-adrenoceptor activity was confirmed in the present study since LY53857 did not displace [^{125}I]HEAT,an α_1-adrenoceptor ligand in autoradiographical studies. Initial studies with LY53857 resulted in complex inhibition of 5-HT-induced contraction in the rat prostate. Contraction to low concentrations of 5-HT appeared inhibited whereas the contractile response to higher concentrations of 5-HT appeared unaffected in the presence of 10 and 30 nM LY53857.

Since 5-HT has been shown to interact with α-

adrenoceptors (Purdy et al., 1987), 5-HT-induced contraction was studied further in the presence of α_1-adrenoceptor blockade with prazosin (1 μM). As observed with several vascular beds (Purdy et al., 1987), the maximum contractile response induced by 5-HT was significantly reduced by approximately 40% in the presence of prazosin (Fig. 3). These data suggest that in the rat prostate, high concentrations of 5-HT induce a contraction that can be attributed to α_1-adrenoceptor activation.

Removal of the α_1-adrenoceptor component of the contractile response to 5-HT with prazosin permitted LY53857 to inhibit competitively the 5-HT contraction of the prostate, with an apparent affinity of LY53857 consistent with its affinity at 5-HT_{2A} receptors ($-\log K_B = 9.02 \pm 0.4$). Although LY53857 also has high affinity at the 5-HT_{2C} receptor, to date, activation of this receptor has not been demonstrated to mediate a contractile response in any peripheral tissue. Therefore, it is unlikely that contraction to 5-HT in the prostate resulted from activation of 5-HT_{2C} receptors.

Thus, in the rat prostate, the contractile response to 5-HT can be attributed both to α_1-adrenoceptor activation and to 5-HT_{2A} receptor activation. Activation of α_1-adrenoceptors may have resulted from a direct activation of α_1-adrenoceptors by 5-HT, or via an indirect effect of 5-HT to enhance norepinephrine release which subsequently activated α-adreno-receptors. If 5-HT were directly activating α-adrenoceptors in prostate smooth muscle, then serotonin should have displaced [^{125}I]HEAT binding in our autoradiographical studies. However, preincubation with 5-HT (10–100 μM) did not significantly reduce [^{125}I]HEAT binding in prostate smooth muscle. The inability of 5-HT to displace [^{125}I]HEAT binding suggests that 5-HT was not directly binding to the HEAT binding site of prostatic α_1-adrenoceptors. Because of the extremely thin layers of smooth muscle in the rat prostate and the relatively strong binding in what appeared to be stromal epithelium, we cannot rule out the possibility that 5-HT might displace [^{125}I]HEAT from a small number of smooth muscle α_1-adrenoceptors. However, based on the overall binding, we feel it is unlikely that the ability of prazosin to inhibit maximum contractile responses to 5-HT occurred because 5-HT directly activated α_1-adrenoceptors.

In summary, autoradiographical studies with [^{125}I]HEAT and [^{125}I]LSD confirmed the presence of α_1-adrenoceptors and 5-HT receptors, respectively, in the rat prostate. Although approximately 50% of the contractile response to 5-HT in the rat prostate results from α_1-adrenoceptor activation, the inability of 5-HT to displace [^{125}I]HEAT in autoradiographical studies suggests that 5-HT may not directly or competitively interact with the α_1-adrenoceptor. Additional studies will be necessary to determine whether activation of α_1-adrenoceptors occurs indirectly through 5-HT-induced stimulation of norepinephrine release, allosteric activation of the α-adrenoceptor by 5-HT, or some additional mechanism. Nevertheless, the present studies raise the intriguing possibility that prostatic 5-HT may contribute both directly (5-HT_2 receptor interaction) and/or indirectly to rat prostatic adrenergic contractility. While rat prostate glands are not structurally identical to human prostate glands, comparisons of other rat tissues to analogous human tissues have predicted the presence of the same 5-HT receptor class. The identification of 5-HT_{2A} (or possibly 5-HT_{2C}) receptors in the rat prostate will facilitate targeted characterization studies of the 5-HT receptors in the human prostate.

Acknowledgment

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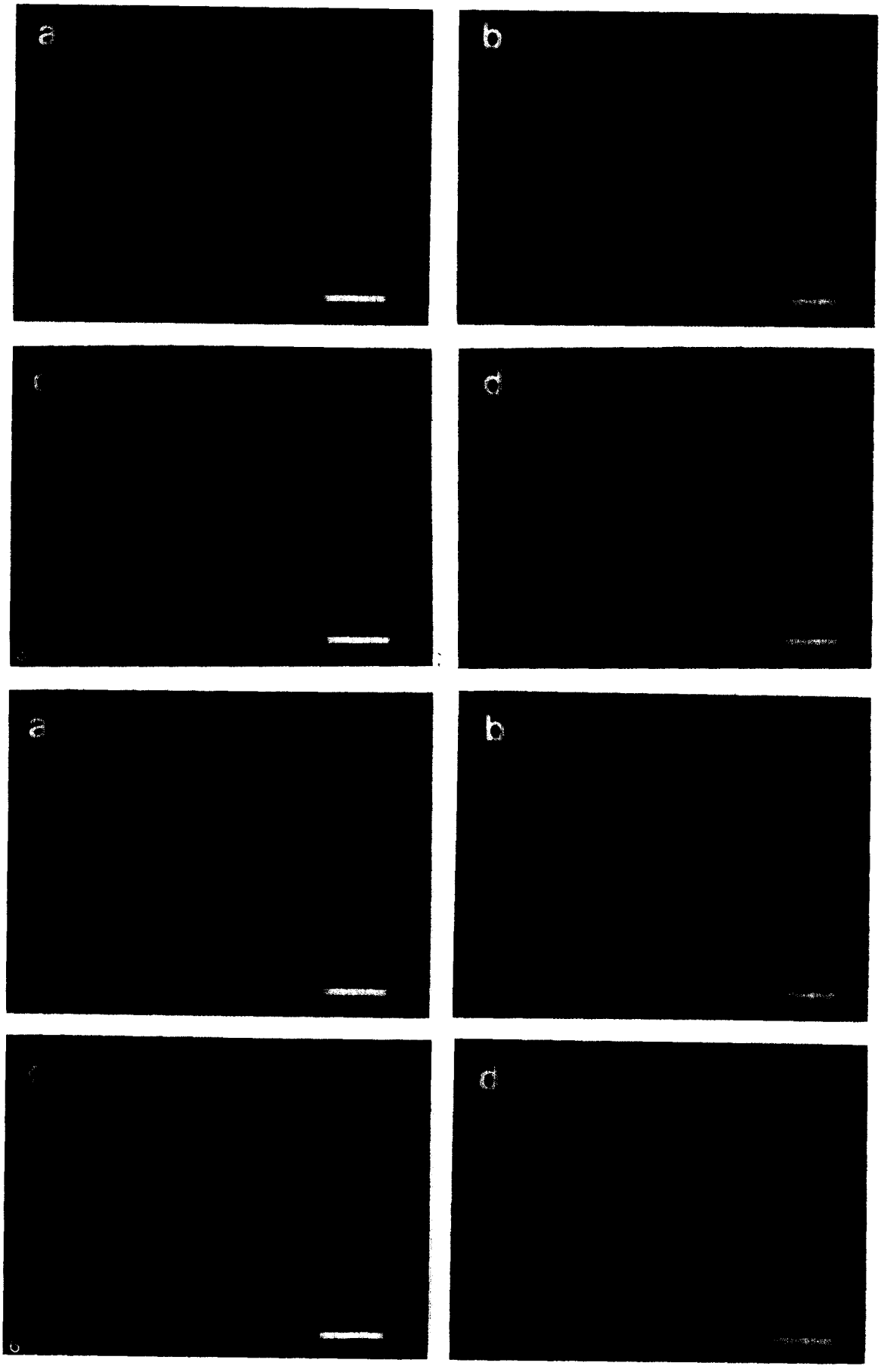
Fig. 5. Representative images of autoradiographic binding studies of [^{125}I]LSD in serial sections of the left ventral lobe rat prostate (cross-section, rostral to caudal) from one animal. Images are color-converted black and white images of ß-Max hyper film autoradiograms produced on the video-based color imaging system MCID (Research Imaging, Ste Catherine's, Ontario, Canada), where high density of silver grains (high exposure) is represented as the color red and low density as violet. Panel (A) is an autoradiogram of 175 pM [^{125}I]LSD binding alone (total binding). Panel (B) is 175 pM [^{125}I]LSD with 1 μM prazosin. Panel (C) is 175 pM [^{125}I]LSD with 10 μM 5-HT. Panel (D) is 175 pM [^{125}I]LSD with 1 μM LY53857. All 4 panels are from the same animal in the same experiment. White bar represents 2 mm. Silver grain densities from these studies are presented in Table 1.

Fig. 6. Representative images of autoradiographic binding studies of [^{125}I]HEAT in serial sections of the left ventral lobe rat prostate (cross-section, rostral to caudal) from one animal. Images are color-converted black and white images of ß-Max hyper film autoradiograms produced on the video-based color imaging system MCID (Research Imaging, Ste Catherine's, Ontario, Canada), where high density of silver grains (high exposure) is represented as the color red and low density as violet. Panel (A) is an autoradiogram of 50 pM [^{125}I]HEAT binding alone (total binding). Panel (B) is 50 pM [^{125}I]HEAT with 1 μM prazosin. Panel (C) is 50 pM [^{125}I]HEAT with 10 μM 5-HT. Panel (D) is 175 pM [^{125}I]HEAT with 1 μM LY53857. All 4 panels are from the same animal in the same experiment. White bar represents 2 mm. Silver grain densities from these studies are presented in Table 2.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