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Expression and pharmacological characterization of α_1-adrenoceptors in rat seminal vesicle

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Abstract

In the present study, we investigated the α_1-adrenoceptor subtypes mediating adrenaline-induced contractions of the rat seminal vesicle by using functional studies. The reverse transcription combined with polymerase chain reaction (RT-PCR) was also used to identify of α_1-adrenoceptor mRNA subtypes. The rank order of potency of α_1-adrenoceptor antagonists in blocking the contractile effects of adrenaline was: prazosin = WB 4101 $\ggg$ BMY 7378 > chloroethylclonidine, indicating the presence of α_{1A}-adrenoceptors in the rat seminal vesicle. In the presence of nifedipine, there was a 76% reduction in the adrenaline-induced contractions. The nifedipine-insensitive component (24%) of the contractile response to adrenaline was unaffected by chloroethylclonidine. A small pool of spare α_1-adrenoceptors for adrenaline (0.10%) was also detected. All three α_1-adrenoceptor subtypes were amplified when RT-PCR was performed on total RNA isolated from rat seminal vesicle. In conclusion, these data indicate the presence of three α_1-adrenoceptor mRNA subtypes, but only α_{1A}-adrenoceptors are involved in the rat seminal vesicle contraction.

Keywords: Seminal vesicle; α_1-Adrenoceptor mRNA subtype; α_{1A}-Adrenoceptor; Adrenaline; Smooth muscle

1. Introduction

Seminal vesicles are a pair of secretory accessory sex glands which consist of a thick outer layer of circular and elongated smooth muscle, stroma and the epithelial cells lining the lumen (Williams-Ashman, 1983). The smooth muscle layers are densely innervated by sympathetic noradrenergic fibers and parasympathetic cholinergic fibers (Wakade and Kirpekar, 1971; Fedan et al., 1977; Yuri, 1990). Previous studies have shown that adrenaline is the most potent α_1-adrenoceptor agonist to induce contractions of the seminal vesicle, by interacting with postsynaptic α_1-adrenoceptors (Sharif et al., 1990; Soares et al., 1993). Presynaptic and/or postsynaptic α_2-adrenoceptors are not present in this organ (Shima, 1993; Sadraei et al., 1995).

Three distinct α_1-adrenoceptor subtypes have been cloned and characterized in tissues of various species: α_{1A}-(Schwinn et al., 1990; Hirasawa et al., 1993; Laz et al., 1994; Perez et al., 1994; Rokosh et al., 1994), α_{1B}-(Cotecchia et al., 1988; Voigt et al., 1990; Ramarao et al., 1992) and α_{1D}-adrenoceptors (Bruno et al., 1991; Lomasney et al., 1991; Perez et al., 1991; see for review Langer, 1998 and Docherty, 1998). Unlike prazosin, which binds to the three α_1-adrenoceptor subtypes with the same affinity, 5-methyl urapidil, WB 4101 and niguldipine show higher affinity for α_{1A}-adrenoceptors. BMY 7378 recognizes preferentially α_{1D}-adrenoceptors (Deng et al., 1996; Saussy et al.,1996; Xin et al., 1997; Yang et al., 1997). Another tool to discriminate α_1-adrenoceptor subtypes is their sensitivity to the alkylating agent chloroethylclonidine (Ford et al., 1994; Hieble et al., 1995; Michel et al., 1995; Graham et al., 1996), an irreversible antagonist that inactivates 80% of both α_{1B} and α_{1D}-adrenoceptors and 20% of the α_{1A}-adrenoceptors (Laz et al., 1994; Hieble et al., 1995; Xiao and Jeffries, 1998). A fourth α_1-adrenoceptor, designated α_{1L}-adrenoceptor, has been characterized in functional but not in molecular studies, and has been proposed to mediate contraction of human, rabbit and dog lower urinary tract tissues (Muramatsu et al., 1994; Ford et al., 1996; Testa et al., 1996; Fukasawa et al., 1998). Its

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relationship to the three fully characterized α_1-adrenoceptors is not known, but there are evidences that this receptor may not derive from a distinct gene, but rather may represent a different conformational state of the α_{1A}-adrenoceptor (Ford et al., 1997).

Many studies have been conducted to identify α_1-adrenoceptor subtypes mediating contraction in male accessory sex organs. A predominant population of α_{1A}-adrenoceptors has been demonstrated in rat vas deferens and prostate (Mallard et al., 1992; Aboud et al., 1993; Yazawa and Honda, 1993; Lepor et al., 1994; Teng et al., 1994; Burt et al., 1995, 1998; Pupo, 1998). It is also known that α_1-adrenoceptors participate in the contraction of the seminal vesicle induced by catecholamines (Soares et al., 1993), but the specific receptor subtypes involved in the contractile response has not been reported yet. The present study was, therefore, designed to characterize the α_1-adrenoceptor subtypes mediating contraction of the rat seminal vesicle, by using functional studies with antagonists exhibiting different selectivity for the α_1-adrenoceptor subtypes. The reverse transcription combined with polymerase chain reaction (RT-PCR) was also used to identify α_1-adrenoceptor mRNA subtypes in the seminal vesicle.

2. Materials and methods

2.1. Animals

Male Wistar rats (60-days-old, 210–220 g) were housed in the Animal Facility at Instituto Nacional de Farmacologia, UNIFESP-EPM and maintained on a 12 h light, 12 h dark lighting schedule, at 20°C, food and water ad libitum.

2.2. Tissue preparation and record of seminal vesicle contraction

The seminal vesicle was dissected, free of connective and fat tissue, separated from coagulating gland and washed internally with a nutrient solution of the following composition (mM): 136.89 NaCl, 5.63 KCl, 1.80 $CaCl_2$, 0.36 NaH_2PO_4, 14.88 $NaHCO_3$ and 5.55 glucose (pH 7.6–7.8) (Picarelli et al., 1962). The organ was suspended, under a load of 0.5 g, into organ baths filled with nutrient solution containing 10^{-6} M propranolol and 10^{-5} M cocaine, bubbled with air, at 30°C. Isometric contractions were recorded with a force-displacement transducer connected to a polygraph (Gemini 7070, Ugo Basile, Viarese, Italy). The tissue was allowed at least 30 min equilibration time prior to the addition of adrenaline. All concentration–effect curves were obtained using the cumulative method of drug addition (Van Rossum, 1963). The interval between curves was 45 min and the nutrient solution was renewed every 10 min. Preliminary experiments, in the absence of antagonists, were carried out and no difference in the sensitivity to adrenaline among five consecutive concentration–effect curves were observed. Thus, after determination of the second concentration–effect curve to adrenaline (control), the agonist was washed out for at least 45 min and then an antagonist was incubated for 45 min. After this period, another concentration–effect curve to adrenaline was obtained. When chloroethylclonidine or phenoxybenzamine was used as antagonist, the tissues were incubated for 30 min or 45 min, respectively, the antagonists were washed out and another concentration–effect curve to adrenaline was obtained.

In another series of experiments, concentration–effect curves to adrenaline were obtained in the absence and presence of nifedipine (10^{-5} M, 30 min) or after incubation with nifedipine (10^{-5} M, 30 min) followed by incubation for further 30 min with chloroethylclonidine (10^{-4} M).

2.3. Data analysis

The data were expressed as percentage of the maximum response to adrenaline obtained in the absence of antagonists (control) and analysed by the interactive nonlinear regression through the computer program GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA). The EC_{50}, concentration that produces 50% of agonist maximum response, was determined.

The antagonist potency was expressed as pA_2 or pK_B values. When the slope was not significantly different from negative unity, the pA_2 value was obtained from the *x*-intercept of the plot of log (agonist concentration-ratio minus one) against log [antagonist concentration] (Arunlakshana and Schild, 1959; Tallarida et al., 1979). When chloroethylclonidine was used, its antagonistic potency was expressed as pK_B, i.e., the negative log of the dissociation constant K_B, which is equal to the molar concentration of the antagonist divided by the concentration-ratio minus one (Besse and Furchgott, 1976).

The active receptor fraction remaining after inactivation by phenoxybenzamine (*q*-value) was determined according to Furchgott and Bursztyn (1967). The reciprocals of equieffective adrenaline concentrations before phenoxybenzamine incubation ($1/[A]$) were plotted against the reciprocals of equieffective adrenaline concentration after partial inactivation of the tissue adrenoceptors by the irreversible antagonist ($1/[A']$). The *q*-value was obtained from the intercept of the straight line with the ordinate. The linear regression for calculation of pA_2 and *q*-values was performed using GraphPad Prism (GraphPad Prim Software, San Diego, CA, USA).

2.4. Determination of α_1-adrenoceptor mRNA by RT-PCR

Total RNA was extracted from frozen seminal vesicles and brain using guanidine isothiocyanate followed by centrifugation through a cesium chloride gradient, as de-

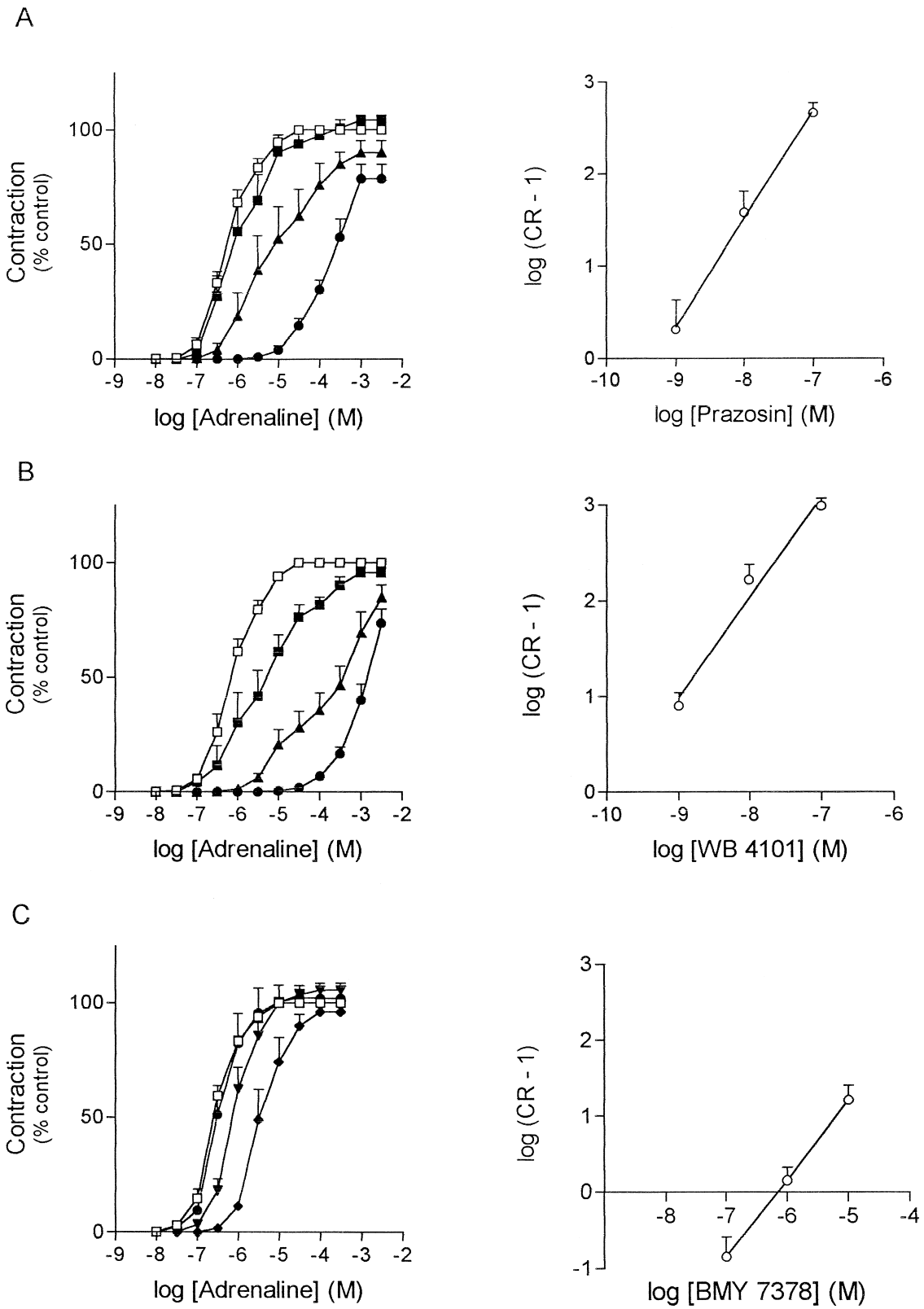

Fig. 1. Effects of prazosin, WB 4101 and BMY 7378 on adrenaline-induced contractions of the rat seminal vesicle. Concentration–effect curves to adrenaline were obtained in the absence (control, □) and presence of α_1-adrenoceptor antagonists (closed symbols). Left panels: (A) prazosin (10^{-9} M, ■; 10^{-8} M, ▲; 10^{-7} M, ●), (B) WB 4101 (10^{-9} M, ■; 10^{-8} M, ▲; 10^{-7} M, ●) and (C) BMY 7378 (10^{-7} M, ●; 10^{-6} M, ▼; 10^{-5} M, ♦). Right panels: Schild plots for α_1-adrenoceptors. Each point and vertical line represent the mean ± S.E.M. of *n* shown in Table 1.

scribed by Chirgwin et al. (1979). RNA samples were then quantitated, using a spectrofotometer at 260 nm/280 nm (Spectronic 2000, Bausch and Lomb, USA) and stored at −70°C for later use.

RT-PCR amplification was performed using SUPERScript™ preamplification system for first strand cDNA synthesis, according to manufacturer's instructions. Reverse transcription of total RNA (5 μg) using oligo(dT)$_{12-18}$ (0.5 μg) was performed in a reaction volume of 20 μl. Reactions in the absence of reverse transcriptase were also included for each RNA tested in order to check for genomic contamination. The resulting cDNA (2 μl) was amplified using GeneAmp RNA PCR kit in a reaction volume of 25 μl containing 1.5 mM $MgCl_2$, 0.2 mM of each primers, 0.5 mM deoxyribonucleotides (dNTPs) and 1.25 U Taq polymerase. Samples were transferred to capillary tubes and PCR amplification performed in an Idaho Rapidcycler (Idaho Technologies, Idaho, USA) are as follows: one cycle of denaturation 96°C, 30 s, followed by 35 cycles of denaturation 96°C, 10 s; annealing 58°C (α_{1a}) or 55°C (α_{1b}, α_{1d}), 10 s; extension 74°C, 40 s (α_{1b}, α_{1d}) or 15 s (α_{1a}). A final extension of 74°C, 2 min was performed for all samples. Aliquots of the DNA samples (10 μl) were loaded onto agarose gels (1.8% agarose) containing ethidium bromide (0.5 μg/ml). PCR products were visualized with fluorescent illumination and photographed.

PCR primers were described by Scofield et al. (1995) and designed so that the PCR product would span the nucleotide sequence of the third intracellular loop, specifically amplifying from transmembrane region 5 to transmembrane region 6 of the rat α_{1a} (Alonso-Llamazares et al., 1993; Laz et al., 1994; Perez et al., 1994), α_{1b} (Voigt et al., 1990) and α_{1d} adrenergic receptor (Lomasney et al., 1991; Perez et al., 1991). Primers were synthesized in UNC Oligonucleotide Facility, University of North Carolina, USA. Primer sequence, corresponding base sites, and the size of the PCR product were as follows: *α_{1a} sense*, GTA GCC AAG AGA GAA AGC CG (628–647) and *α_{1a} antisense*, CAA CCC ACC ACG ATG CCC AG (820–839) (Δ 212 bp); *α_{1b} sense*, GCT CCT TCT ACA TCC CGC TCG (629–649), *α_{1b} antisense*, AGG GGA GCC AAC ATA AGA TGA (908–928) (Δ 300 bp); *α_{1d} sense*, CGT GTG CTC CTT CTA CCT ACC (759–779) and *α_{1d} antisense*, GCA CAG GAC GAA GAG ACC CAC (1042–1062) (Δ 304); *GAPDH sense*, CGG GAA GCT TGT GAT CAA TGG (258–277) and *GAPDH antisense*, GGC AGT GAT GCC ATG GAC TG (614–595) (Δ 357). All primers and PCR conditions were tested using total RNA from rat brain, since α_{1a}, α_{1b} and α_{1d} mRNA transcripts are known to be expressed in this tissue.

2.5. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical analysis was carried out using analysis of variance (ANOVA) followed by Newman–Keuls test for multiple comparisons, or by the two-tailed Student's *t*-test to compare two response (Snedecor and Cochran, 1980). *P*-values < 0.05 were accepted as significant.

2.6. Drugs

The following drugs were used: adrenaline (L-epinephrine bitartrate), prazosin (1-[4-amino-6,7-dimethoxy-2-quinazolinyl]-4-[2-furanylcarbonyl]piperazine hydrochloride), propranolol (DL-propranolol hydrochloride) and nifedipine were from Sigma (St. Louis, MO, USA). WB 4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride), BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride), chloroethylclonidine (chloroethylclonidine dihydrochloride) and phenoxybenzamine (phenoxybenzamine hydrochloride) were from Research Biochemicals International (Natrick, MA, USA). Cocaine (cocaine hydrochloride) was from Merck Darmstadt (FRG). SUPERScript™ preamplification system for first strand cDNA synthesis was from Gibco (Gaithersburg, MD, USA). GeneAmp RNA PCR kit was from Perkin-Elmer (Norwalk, CT, USA). All other drugs and reagents were from Sigma.

3. Results

3.1. Functional studies

Prazosin (nonselective α_1), WB 4101 (selective α_{1A}) and BMY 7378 (selective α_{1D}) produced concentration-dependent parallel rightward shifts of the concentration–effect curves to adrenaline (Fig. 1). Schild plots were constructed from the effects of a range of concentrations of each antagonist (Fig. 1). Since the slope of these regressions did not differ significantly from the negative unity, the pA_2 values were calculated and summarized in Table 1.

Chloroethylclonidine 10^{-5} M and 3×10^{-5} M shifted concentration–effect curves to adrenaline three- and nine-fold to the right, respectively, with no change in the maximum response to adrenaline. Chloroethylclonidine 10^{-4} M shifted concentration–effect curves to adrenaline

Table 1
pA_2 values for α_1-adrenoceptor antagonists against adrenaline-induced contractions in the rat seminal vesicle. Data are mean ± S.E.M. of number of experiments in parenthesis

Antagonist	pA_2 value	Slope
Prazosin	9.52 ± 0.35 (5)	1.17 ± 0.09
WB4101	9.96 ± 0.20 (6)	1.16 ± 0.08
BMY7378	6.18 ± 0.19 (4)	0.91 ± 0.07

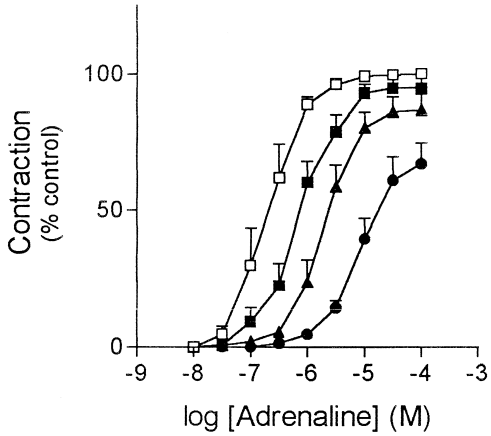

Fig. 2. Effect of chloroethylclonidine on adrenaline-induced contractions of the rat seminal vesicle. Concentration–effect curves to adrenaline were obtained in the absence (control, □) and presence of chloroethylclonidine (10^{-5} M, ■; 3×10^{-5} M, ▲; 10^{-4} M, ●). Each point and vertical line represent the mean ± S.E.M. of five experiments.

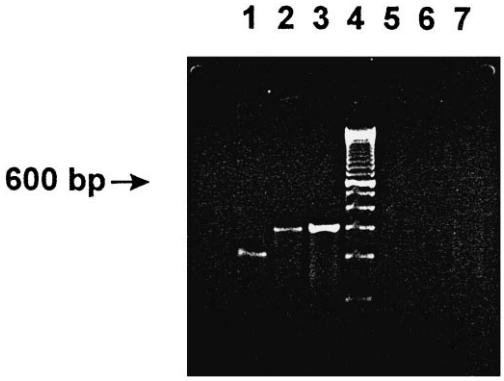

Fig. 4. Gel electrophoresis of RT-PCR products amplified from total seminal vesicle RNA. The RT-PCR was carried out as described in Section 2. DNA products from PCR were resolved on 1.8% agarose gel and visualized by ethidium bromide staining. Size molecular markers are indicated, with bands labeled in base pairs. Messages for α_{1a}, α_{1b} and α_{1d} were apparent (lanes 1, 3 and 5). The negative RT-PCR control for each α_1 mRNA subtype, run in parallel, contained all the components except reverse transcriptase (lanes 2, 4 and 6 for α_{1a}, α_{1b} and α_{1d}, respectively).

35-fold to the right and reduced 43% of the contractile maximum response induced by adrenaline (Fig. 2). The calculated pK_B value for each concentration of chloroethylclonidine was 5.09 ± 0.20, 5.35 ± 0.35 and 5.24 ± 0.15, respectively. The pA_2 and pK_B values calculated for each antagonist tested showed that the order of potency for α_1-adrenoceptor antagonists in the rat seminal vesicle was prazosin = WB 4101 ⋙ BMY 7378 > chloroethylclonidine.

The irreversible α_1-adrenoceptor antagonist phenoxybenzamine (5×10^{-10} M, 10^{-9} M and 5×10^{-9} M) reduced the contractile maximum response to adrenaline by 42%, 70% and 84%, respectively (Fig. 3A). When the plot of reciprocals of the equieffective adrenaline concentration before and after phenoxybenzamine incubation was obtained (Fig. 3B), the experimental data fitted a straight line, allowing the calculation of the *q*-value. The results suggested that 0.10% of the α_1-adrenoceptor pool represents a spare receptor population for adrenaline in the rat seminal vesicle.

The maximal contractions induced by adrenaline in the seminal vesicle were reduced 76.40 ± 4.50% ($n = 4$ experiments) ($P < 0.05$) when tissues were incubated with

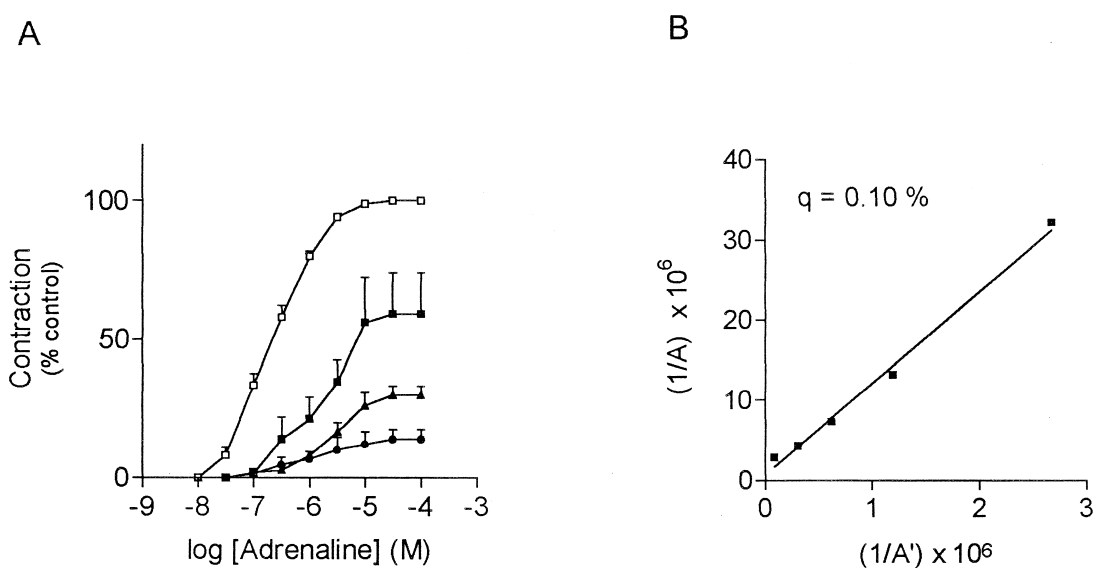

Fig. 3. Effect of phenoxybenzamine on adrenaline-induced contractions of the rat seminal vesicle. (A) Concentration–effect curves to adrenaline were obtained in the absence (control, □) and presence of phenoxybenzamine (5×10^{-10} M, ■; 10^{-9} M, ▲; 5×10^{-9} M, ●). Each point and vertical line represent the mean ± S.E.M. of three experiments. (B) Plot of reciprocals of equieffective adrenaline concentrations before (1/A) and after (1/A′) phenoxybenzamine treatment. *q*-value is the active receptor fraction remaining after receptor inactivation by phenoxybenzamine.

nifedipine 10^{-5} M for 30 min. The addition of chloroethylclonidine (10^{-4} M) for further 30 min did not change significantly the nifedipine-induced reduction in the maximum response to adrenaline (63.70 ± 8.70%, $n = 4$ experiments, $P < 0.05$).

3.2. Identification of mRNA α_1-adrenoceptor subtypes in seminal vesicle

All three α_1-adrenoceptor subtypes were amplified when RT-PCR was performed on total RNA from seminal vesicle (Fig. 4, lanes 1, 2 and 3). The primers were able to amplify α_{1a}, α_{1b} and α_{1d} cDNAs, and each PCR product was of the predicted size. No PCR products were detected when reverse transcriptase was omitted from the RT-PCR reaction, demonstrating that the amplified products were from cDNA and not from genomic DNA (Fig. 4, lanes 5, 6 and 7).

4. Discussion

The present study investigated the pharmacological α_1-adrenoceptor subtypes involved in the contractile response of the rat seminal vesicle. Although α_{1a}, α_{1b}, and α_{1d} adrenoceptor transcripts were detected by using RT-PCR technique, functional studies indicated that α_{1A}-adrenoceptor subtype is predominantly involved in the contractile response.

Prazosin competitively antagonized adrenaline-induced contractions in the seminal vesicle, with pA_2 value (9.52 ± 0.35) consistent for α_1-adrenoceptors and similar to those obtained in rat epididymal vas deferens, spleen and portal vein (pA_2 = 9.2) (Burt et al., 1995; Marshall et al., 1996) and rat aorta (pA_2 = 9.8) (Kenny et al., 1995). WB 4101 was also a competitive antagonist in this tissue and had relatively high affinity (pA_2 = 9.96 ± 0.20), consistent with that found for α_{1A}-adrenoceptors in rat vas deferens (Aboud et al., 1993; Burt et al., 1995) and human prostate (Marshall et al., 1995), while the α_{1D}-subtype selective antagonist, BMY 7378, had a relatively low affinity (pA_2 = 6.18 ± 0.19) compared with that expected for the α_{1D}-subtype (Goetz et al., 1995; Kenny et al., 1995; Deng et al., 1996; Saussy et al., 1996), indicating that functional α_{1D}-adrenoceptor may not be involved in the seminal vesicle contracticle response to adrenaline.

We tested the pA_2 values obtained in this study against the average pK_i values, reported for the different α_1-adrenoceptor antagonists, obtained in several binding studies on cell line membranes expressing either the cloned

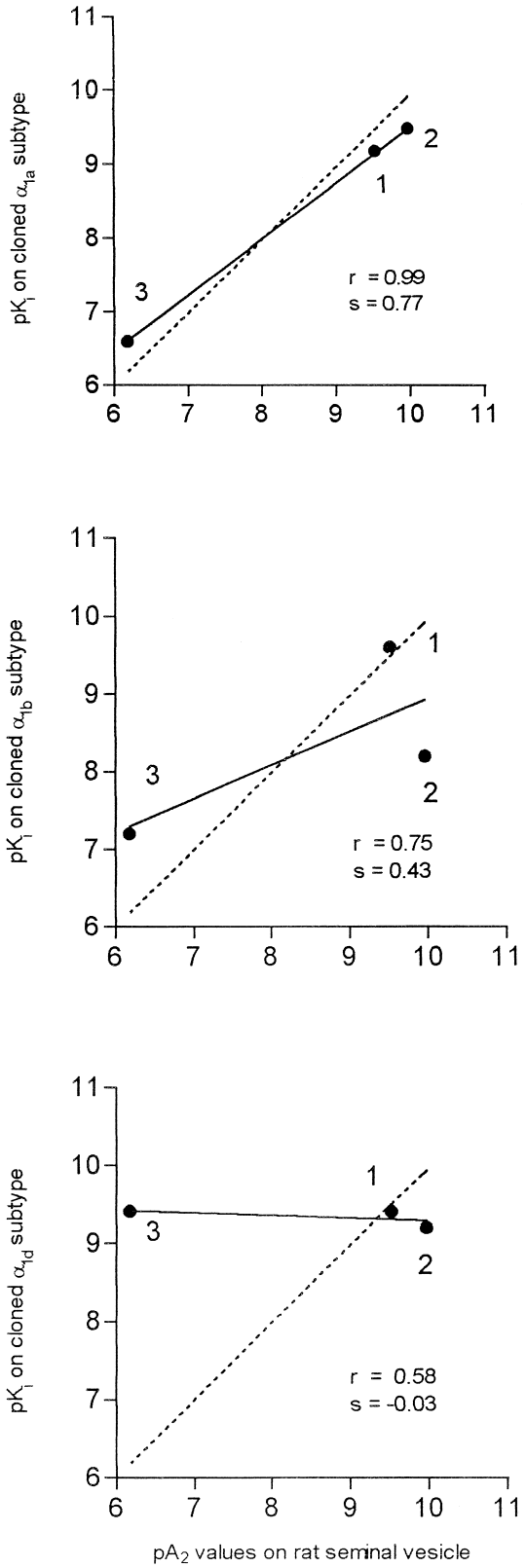

Fig. 5. Correlation of average pK_i values for the displacement of [^{3}H]prazosin on cloned α_1-adrenoceptor subtypes (from Faure et al., 1994; Forray et al., 1994; Kenny et al., 1994a,b; Testa et al., 1994 and Goetz et al., 1995, summarized by Burt et al., 1995) with pA_2 values obtained in functional studies for the antagonists prazosin (1), WB 4101 (2) and BMY 7378 (3). The solid line is the linear regression fit through all the points. The dashed line has a slope equal to unity, passing through the origin. r = correlation coefficient and s = slope.

α_{1a}, α_{1b} or α_{1d}-adrenoceptors (Faure et al., 1994; Forray et al., 1994; Kenny et al., 1994a,b; Testa et al., 1994 and Goetz et al., 1995, summarized by Burt et al., 1995). The analysis of these data showed that the most significant correlation was obtained when pA_2 values in the seminal vesicle were compared with pK_i values reported for the α_{1A}-cloned adrenoceptor subtype (Fig. 5), indicating that pharmacological α_{1A}-adrenoceptors is predominantly involved in adrenaline-induced contraction in this organ.

In order to further characterize functional α_1-adrenoceptors involved in seminal vesicle contraction, we tested the sensitivity of seminal vesicle contractions induced by adrenaline to the alkylating agent chloroethylclonidine, an irreversible antagonist which inactivates 80% of both α_{1B}- and α_{1D}-and 20% of α_{1A}-adrenoceptors. Our results showed that chloroethylclonidine at the highest concentration (10^{-4} M) produced only a 35-fold shift to the right in the adrenaline-induced curves, with 43% reduction in the maximum contractile response. The results were not consistent with those reported by Burt et al. (1995) in the rat spleen, a tissue that contains mainly α_{1B}-adrenoceptors. It was showed that chloroethylclonidine (10^{-4} M) produced a 300-fold shift to the right in phenylephrine-induced concentration–effect curves, with 60% reduction in the maximum response. Thus, the effects of chloroethylclonidine on seminal vesicle can not be directly correlated to the presence of α_{1B}- or α_{1D}-adrenoceptors.

It is known that the degree to which chloroethylclonidine affects a functional response depends on some extent on the amount of spare receptors present in the tissue (Burt et al., 1995; Marshall et al., 1995). Thus, we also tested the effects of the irreversible α_1-adrenoceptors antagonist phenoxybenzamine on the seminal vesicle contractile response to adrenaline and the results indicated that only 0.10% of the α_1-adrenoceptor pool represents a spare receptor population in this tissue. This may explain why contraction of the rat seminal vesicle to adrenaline is more sensitive to chloroethylclonidine when compared with rat vas deferens (Minneman et al., 1988; Burt et al., 1995), a tissue with large spare receptors (Díaz-Toledo and Martí, 1988), and with human prostate (Teng et al., 1994; Marshall et al., 1995). It is worth-noting that the corresponding expressed clones for α_{1A}-subtype have also been shown to be partially chloroethylclonidine-sensitive (Laz et al., 1994; Xiao and Jeffries, 1998).

In the presence of the 1,4-dihydropiridine-type Ca^{+2} channel blocker, nifedipine, the contraction induced by adrenaline in the rat seminal vesicle was reduced by 76%. Similar results were obtained in rat vas deferens (Aboud et al., 1993) and guinea-pig cauda epididymis (Haynes and Hill, 1996). On the other hand, the nifedipine-insensitive component (25%) of the seminal vesicle contractile response to adrenaline was unaffected by chloroethylclonidine, as previously shown in the rat vas deferens (Teng et al., 1994; Burt et al., 1995). Taken together, the effects of α_1-adrenoceptor antagonists and the effect of nifedipine, our results indicate that the contraction of the seminal vesicle is mediated by α_{1A}-adrenoceptor subtype and that the effector mechanisms involved in this contraction are predominantly linked to influx of extracellular Ca^{+2}.

In fact, RT-PCR studies confirmed that α_{1A} transcripts are present in rat seminal vesicle. It is important to note, however, that mRNA for α_{1b} and α_{1d} adrenoceptor subtypes were also identified in this tissue. Although the level of receptor mRNA in a given tissue may not always correlate directly with the levels of receptor protein, and taking into consideration that different cell types are present in seminal vesicle (Williams-Ashman, 1983), further studies will be necessary to understand the relative importance of these transcripts for the seminal vesicle. Functional studies, using moderately selective drugs, coupled with localization and determination of the relative levels of receptor subtypes by using immunohistochemical techniques could be an important tool to better characterize α_1-adrenoceptor subtypes present in the rat seminal vesicle.

In conclusion, the present study demonstrates the presence of three different α_1-adrenoceptor mRNA transcripts (α_{1a}, α_{1b} and α_{1d}), but only α_{1A}-adrenoceptors are involved in the rat seminal vesicle contraction.

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