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European Journal of Pharmacology 443 (2002) 151–168

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Affinity profile at α_1- and α_2-adrenoceptor subtypes and in vitro cardiovascular actions of (+)-boldine

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Received 4 February 2002; received in revised form 27 March 2002; accepted 5 April 2002

Abstract

The present study examines the functional and binding affinities of the aporphine alkaloid, (+)-boldine, at different α_1- and α_2-adrenoceptor subtypes, namely, α_{1A} (rat vas deferens and kidney) and its L-like state (rabbit spleen), α_{1B} (guinea pig spleen, mouse spleen and rabbit aorta), α_{1D} (rat aorta and pulmonary artery), at possible subtypes of prejunctional α_2-adrenoceptors in rat and rabbit vas deferens and rat atrium, α_{2D} in guinea pig ileum, cloned human α_1-adrenoceptor subtypes A, B and D and α_2-adrenoceptor subtypes A, B and C as well as rat α_{2D}-adrenoceptors. Additionally, we investigated its Ca^{2+} channel antagonism in vascular and cardiac preparations. (+)-Boldine had higher affinity at α_1-adrenoceptor subtype A ($pA_2 = 7.46$, $pK_i = 7.21$) compared with its L-like state ($pA_2 = 5.63$) or subtype B ($pA_2 = 5.98 - 6.12$, $pK_i = 5.79$) and subtype D ($pA_2 = 6.18 - 6.37$, $pK_i = 6.09$). Its affinities at α_2-adrenoceptors in rat and rabbit vas deferens and rat atrium ($pA_2 = 6.02$, 6.36, 6.06, respectively) were identical, but lower at guinea pig ileum α_{2D}-adrenoceptors ($pA_2 = 4.38$). (+)-Boldine displayed nearly undistinguishable affinity at cloned human α_2-adrenoceptor subtypes A, B and C ($pK_i = 6.26$, 5.79 and 6.35, respectively), whereas its affinity at rat α_{2D}-adrenoceptors was low ($pK_i = 4.70$). In perfused rat kidney, (+)-boldine inhibited K^+-evoked vasoconstriction at doses 70-fold higher than diltiazem. In guinea pig Langendorff heart, (+)-boldine ($10^{-5} - 2 \times 10^{-4}$ M) was equieffective in increasing coronary flow and in depressing cardiac force, while lower concentrations already depressed heart rate. In papillary muscles from guinea pig, (+)-boldine ($10^{-6} - 10^{-5}$ M) mainly prolonged the duration of action potential at levels >30% of repolarization. These data reveal that (+)-boldine, except for its moderate selectivity (15 to 25-fold) for α_{1A}-adrenoceptors, does not discriminate between the α_1-adrenoceptor subtypes B and D and α_2-adrenoceptor subtypes A, B and C, at which the drug consistently displays micromolar affinity. In vascular and cardiac preparations, (+)-boldine, although being at least 50-fold weaker than diltiazem, shows Ca^{2+} channel antagonistic properties but no specificity for coronary dilatation relative to cardiodepression.

Keywords: (+)-Boldine; α_1-Adrenoceptor; α_2-Adrenoceptor; Subtype; Selectivity; Functional experiment; Radioligand binding study; Ca^{2+} channel antagonism; Cardiac effect

1. Introduction

Three distinct subtypes of the α_1-adrenoceptor (α_{1A}, α_{1B} and α_{1D}) have been identified by both classical pharmacological and molecular biological techniques in various tissues (Hieble et al., 1995; Michel et al., 1995). Additionally, the existence of a fourth α_1-adrenoceptor, designated α_{1L} and representing a functional state of the α_{1A}-adrenoceptor (Ford et al., 1997), has been postulated to mediate a contraction in some tissues (Muramatsu et al., 1990, 1998; Ford et al., 1996; Kenny et al., 1996; Testa et al., 1997). A number of antagonists selective for these subtypes and suitable for their characterization in different tissues are now available, e.g. RS-17053, Rec 15/2739 and B8805-033 for subtype A (Ford et al., 1996; Testa et al., 1997; Eltze et al., 2001a), L-765,314 for subtype B (Chang et al., 1998), or BMY 7378, MDL 73005EF and A-315456 for subtype D (Goetz et al., 1995; Saussy et al., 1996; Buckner et al., 2001). Similarly, based on ligand binding and molecular cloning studies, α_2-adrenoceptors have been subdivided in A, B, C and D; however, mainly due to paucity of sufficiently selective ligands, some controversy still remains regarding some assignments of function to one of these subtypes (Bylund et al., 1988, 1994; Alberts, 1993; Trendelenburg et al., 1997).

Apart from the above-mentioned and more known α_1-adrenoceptor subtype-selective antagonists, a benzylisoqui-

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01591-1

nolone alkaloid structurally related to aporphine, (+)-boldine ((*S*)-2,9-dihydroxy-1,10-dimethoxy-aporphine), has been shown to discriminate between α_{1A}- and α_{1B}-adrenoceptors (Ivorra et al., 1995). (+)-Boldine can be isolated from the leaves and bark of boldo (*Peumus boldus* Molina, Monimiaceae), a Chilean tree traditionally employed in folk medicine and used as a medicinal plant for the treatment of digestive and hepatobiliary disorders. The chemical and pharmacological properties of the alkaloid have been summarized earlier (Speisky and Cassels, 1994). Its main pharmacological actions comprise antioxidative and cytoprotective properties (Speisky et al., 1991a; Cederbaum et al., 1992; Bannach et al., 1996), blockade of α_1-adrenoceptors and Ca^{2+} channels (Ivorra et al., 1993a,b), and a neuroleptic-like action in mice (Zetler, 1988), suggesting that it may also act as dopamine antagonist (Asencio et al., 1999). Particularly in binding studies, (+)-boldine has been shown to have an up to 65-fold higher affinity for the rat cortical α_{1A}-($pK_i = 7.6–8.3$) than for the α_{1B}-adrenoceptor ($pK_i = 6.5$) (Ivorra et al., 1995; Madrero et al., 1996). Functional studies on the vasorelaxant mechanism in vitro and on the hypotensive activity in anaesthetized rats have revealed predominant α_1-adrenoceptor antagonistic and weak Ca^{2+} entry blocking properties of (+)-boldine (Ivorra et al., 1993a; Chulia et al., 1996; Orallo et al., 1998; Fabeiro et al., 2000). However, except for its ability to antagonize vasoconstriction to α_1-adrenoceptor stimulation in rat aorta ($pA_2 = 6.8–7.1$; Fabeiro et al., 2000) or guinea pig aorta ($pA_2 = 5.64$; Chulia et al., 1996), and to block prejunctional α_2-adrenoceptors in rat vas deferens ($pA_2 = 6.0$; Fabeiro et al., 2000), further functional studies for characterizing the ability of (+)-boldine to interact with different subtypes of the α_1- and α_2-adrenoceptor or to differentially block vascular and cardiac Ca^{2+} channels (coronary dilatation vs. cardiodepressive actions) are missing.

The present study assessed the presumed selectivity of (+)-boldine at different α_1-adrenoceptor subtypes in functional experiments, namely, rat vas deferens and perfused kidney for subtype A (Han et al., 1987; Eltze et al., 1991; Eltze and Boer, 1992), rabbit spleen as a model for the α_{1A}-adrenoceptor existing in its functional α_{1L}-like state (Ford et al., 1997; Oriowo, 1998), guinea pig spleen, mouse spleen and rabbit aorta for subtype B (Eltze, 1994, 1996; Muramatsu et al., 1998) and rat aorta and pulmonary artery for subtype D (Kenny et al., 1995; Hussain and Marshall, 1997; Eltze et al., 1999). While reliable functional correlates of α_{2A}-, α_{2B}-, α_{2C}- and α_{2D}-adrenoceptor binding sites have proved difficult to find and are subject to permanent controversy, we investigated the ability of (+)-boldine to interact with α_2-adrenoceptors located at adrenergic nerve endings in rat and rabbit vas deferens, the former of which has been shown to resemble subtype A, and at the putative subtype B in rat atrium (Alabaster et al., 1986; Connaughton and Docherty, 1990; Smith et al., 1992; Alberts, 1993), which can be differentiated by the $\alpha_{2A/D}$- and α_{2B}-adrenoceptor selective antagonists BRL 44408 and ARC 239, respectively (Bylund et al., 1988, 1994). Additionally, we used the guinea pig ileum longitudinal muscle strip, suggested as a functional assay for subtype D located on cholinergic nerve endings to modulate acetylcholine release (Funk et al., 1995; Colucci et al., 1998). These experiments were completed with radioligand binding studies at cloned human α_1-adrenoceptor subtypes A, B and D stably transfected in Chinese hamster ovary (CHO) cells (Keffel et al., 2000) and α_2-adrenoceptor subtypes A, B and C stably transfected in human embryonic kidney (HEK) cells, as well as at rat cortical α_{2D}-adrenoceptors (Erdbrügger et al., 1995). Moreover, in order to get further insight into the functional strength of (+)-boldine to block Ca^{2+} channels, we evaluated its vascular, cardiac and electrophysiological effects in perfused rat kidney and guinea pig Langendorff heart as well as in guinea pig papillary muscle, in comparison with diltiazem, a Ca^{2+} entry blocker with vasodilator and cardiodepressant activity (Boddeke et al., 1987).

2. Materials and methods

2.1. Rat vas deferens and perfused kidney: α_{1A}-adrenoceptors

Prostatic portions of vas deferens taken from Wistar rats (180–250 g) were set up in 20 ml organ baths containing Tyrode solution plus 10^{-5} M cocaine, maintained at 37 °C and gassed with a mixture of 95% O_2–5% CO_2. Concentration–response curves of isotonic contractions to cumulatively added noradrenaline were performed in the absence or presence of the antagonist equilibrated with the tissue for 20 min (Eltze et al., 1991).

The potency of α_1-adrenoceptor antagonists to attenuate noradrenaline-evoked vasoconstriction was evaluated in isolated kidneys taken from male normotensive Wistar rats (390–420 g) perfused at a constant pressure of 100 cm H_2O with Tyrode (37 °C, gassed with 95% O_2–5% CO_2) containing 6×10^{-7} M noradrenaline, which reduced renal perfusion flow by about 70–80% as previously described (Eltze et al., 1991). Similarly, the vasodilatory effect of injected increasing doses of (+)-boldine in comparison with diltiazem was evaluated in rat kidneys continuously perfused with 27 mM KCl (with concomitant omission of equimolar NaCl), which reduced perfusion flow between 70% and 80%. The percent inhibition of vasoconstriction following the injection of increasing doses of the antagonist (100 μl aqueous bolus within 2 s) was calculated for the determination of their half-maximal vasodilatory effect ($-\log ED_{50}$ mol) (Eltze et al., 1991).

2.2. Rabbit spleen: α_{1L}-like state of α_{1A}-adrenoceptors

Longitudinal splenic strips ($2 \times 2 \times 15$ mm) taken from male New Zealand White rabbits (2.5–3.0 kg, killed by exsanguination after the animals had been anaesthetized with pentobarbital sodium, 60 mg/kg i.v.) were set in

10 ml organ baths under 1 g tension containing the modified Krebs–Ringer bicarbonate buffer used for guinea pig and mouse spleen, additionally containing 10^{-7} M yohimbine to block α_2-adrenoceptors (Eltze et al., 1999). Antagonist affinities were determined from cumulative concentration–response curves of noradrenaline in the absence and presence of antagonists equilibrated with the tissue for 30 min (Oriowo, 1998).

2.3. Guinea pig spleen, mouse spleen and rabbit aorta: α_{1B}-adrenoceptors

Spleens obtained from male guinea pigs (350–400 g) or male mice (25–30 g) were longitudinally cut into six and two strips, respectively, and were set up in 10 ml organ baths under a resting tension of 1.0 and 0.8 g, respectively, for recording isometric contractile responses in Krebs–Ringer bicarbonate buffer maintained at 37 °C and gassed with 95% O_2–5% CO_2, additionally containing 3×10^{-7} M desipramine, 3×10^{-5} M corticosterone and 10^{-6} M propranolol. The contractions in response to cumulative administration of noradrenaline were generated in the absence or presence of antagonists equilibrated with the splenic strips for 30 min as previously described (Eltze, 1994, 1996).

Similarly, ring preparations of the thoracic aorta from male New Zealand White rabbits (see above) were mounted in organ baths under a resting tension of 1.5 g in Krebs–Ringer bicarbonate buffer maintained at 37 °C and gassed with 95% O_2–5% CO_2, additionally containing 3×10^{-7} M desipramine, 3×10^{-5} M corticosterone, 10^{-6} M propranolol and 10^{-7} M yohimbine. Isometric contractions in response to cumulatively added noradrenaline were performed in the absence or presence of antagonists (30 min) after three repetitions of the control curves (Muramatsu et al., 1998; Eltze et al., 2001b).

2.4. Rat thoracic aorta and pulmonary artery: α_{1D}-adrenoceptors

Ring preparations from the thoracic aorta and pulmonary artery of male Wistar rats (350–400 g) were mounted in 10 ml organ baths under a resting tension of 1 g in Krebs–Ringer bicarbonate buffer maintained at 37 °C and gassed with 95% O_2–5% CO_2, additionally containing 3×10^{-7} M desipramine, 3×10^{-5} M corticosterone, 10^{-6} M propranolol and 10^{-7} M yohimbine. Isometric contractions in response to cumulatively added noradrenaline or buspirone were performed in the absence or presence of antagonists equilibrated with the tissue for 30 min (Eltze and Boer, 1992; Eltze et al., 1999).

2.5. Field-stimulated rabbit and rat vas deferens: prejunctional α_2-adrenoceptors

The vasa deferentia from male New Zealand White rabbits (see above) were removed. Two prostatic portions of 1 cm in length were folded over a platinum electrode in 10 ml organ baths and connected to force-displacement transducers under a resting tension of 0.75 g. A second platinum ring electrode was placed at the top of the bathing fluid. The bathing fluid (mM: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.0) was kept at 31 °C and aerated with 95% O_2–5% CO_2. Neurogenic twitch contractions in response to field stimulation (0.05 Hz, 0.5 ms, 30 V), and their change after cumulative administration of UK 14.304 (10^{-10}–3×10^{-8} M) in the absence and presence of antagonist equilibrated with the tissue for 30 min, were measured isometrically (Alabaster et al., 1986).

Similarly, male Wistar rats (200–250 g) were killed by a blow on the head, and after that the vasa deferentia were removed and divided into two. Four preparations from one animal were mounted in 10 ml organ baths under a resting tension of 0.5 g in the bathing solution mentioned for rabbit vas deferens, additionally containing 4×10^{-5} M corticosterone, 10^{-8} M desipramine and 10^{-7} M propranolol, which was kept at 31 °C and aerated with 95% O_2–5% CO_2. Neurogenic contractions in response to field stimulation (0.1 Hz, 3 ms, 30 V) and their inhibition by UK 14.304 (10^{-9}–10^{-7} M) in the absence and presence of antagonist equilibrated with the tissue for 30 min were measured isometrically (Doxey et al., 1977).

2.6. Sympathetic nerve-stimulated rat left atrium: prejunctional α_2-adrenoceptors

Isolated left atria from male Wistar rats (250–350 g) were mounted horizontally in 1 ml organ baths and superfused at 10 ml/min by 34 °C with Krebs solution of (mM) NaCl 118.0, KCl 3.4, $CaCl_2$ 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.5 and Na pyruvate 2.0, aerated with a mixture of 95% O_2–5% CO_2. The initial tension was set at 0.5 g and kept reasonably constant throughout the experiment. The atria were paced by rectangular pulses (2 Hz, 0.2 ms, 10–20 V) by platinum electrodes, one impaled directly at one side of the tissue, the other located at 0.5-mm distance from it at the bottom of the perfusion chamber. After 45 min of equilibration, the sympathetic nerves were excited by field stimulation of high voltage (15 Hz, 1 ms, 80 V, for 0.5 s at intervals of 4 min) through the same platinum electrodes. Following the establishment of consistent inotropic responses, cumulative concentrations of UK 14.304 (10^{-9}–3×10^{-7} M) were administered, which caused a concentration-dependent inhibition of the inotropic response to sympathetic nerve stimulation carried out in the absence or presence (30 min) of the antagonist (Hieble and Pendleton, 1979; Ruffolo et al., 1987).

2.7. Field stimulated guinea pig ileum: prejunctional α_{2D}-adrenoceptors

Longitudinal muscle strips were prepared from the ileum of male guinea pigs (400 –450 g) according to the method

of Paton and Vizi (1969). Briefly, the strips were set up under a resting tension of 1 g in organ baths at 37 °C containing Krebs solution (mM: NaCl 118, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.2, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and choline chloride 0.02) aerated with 95% O_2–5% CO_2. Recurrent phasic contractions of the longitudinal muscle (twitch responses) were evoked by field stimulation by platinum electrodes (0.1 Hz, 0.5 ms, 60 V) and their inhibition by UK 14.304 (10^{-8}–10^{-6} M) in the absence and presence of antagonists equilibrated with the strips for 30 min was measured by force-displacement transducers.

2.8. Cloned human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors

Human α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors stably expressed in Chinese hamster ovary (CHO) cells were identified using [^{3}H]prazosin as the radioligand as previously described (Keffel et al., 2000). Briefly, experiments were performed in binding buffer consisting of 50 mM Tris, 10 mM $MgCl_2$ and 0.5 mM EDTA at pH 7.5 in a total assay volume of 1 ml. The protein content typically was 40–60 μg/assay. The mixtures were incubated at 25 °C for 45 min and incubations were terminated by rapid vacuum filtration over Whatman GF/C filters followed by two washes of the filters each with 10 ml of ice-cold incubation buffer. Non-specific binding which amounted to 5–10% was defined as binding in the presence of 10^{-5} M phentolamine. In competition experiments a single concentration of the radioligand was competed for a high number of narrowly spaced concentrations of (+)-boldine.

2.9. Cloned human α_{2A}-, α_{2B}- and α_{2C}- and rat α_{2D}-adrenoceptors

Similarly, human embryonic kidney (HEK) cells, which had been stably transfected with human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors, and rat cerebral cortex as a source of α_{2D}-adrenoceptors, i.e. the rat homologue of the α_{2A}-adre noceptor, were used to determine the (+)-boldine's affinity to α_2-adrenoceptor subtypes. Competition binding using [^{3}H]RX 821002 as the radioligand was performed as previously described (Erdbrügger et al., 1995). Briefly, experiments were performed in binding buffer consisting of 50 mM Tris, 10 mM $MgCl_2$ and 0.5 mM EDTA at pH 7.5 in a total assay volume of 250 μl. The mixtures were incubated at 25 °C for 60 min and terminated by rapid vacuum filtration over Whatman GF/C filters followed by two washes of the filters each with 10 ml of ice-cold incubation buffer. Non-specific binding was defined as binding in the presence of 10^{-5} M phentolamine.

2.10. Guinea pig Langendorff heart: cardiac effects

Male guinea pigs (400–500 g) were killed by cervical dislocation. The hearts were rapidly excised and perfused with Krebs–Henseleit solution at a constant pressure of 80 cm H_2O (62 mm Hg) via retrograde cannulation of the aorta in a Langendorff apparatus. The perfusate was prewarmed to 37 °C and gassed with a mixture of 95% O_2–5% CO_2. The Krebs–Henseleit solution consisted of (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 5.0. A water-filled balloon catheter connected to a Statham P 23 Db pressure transducer was advanced via the left atrium into the left ventricle and preloaded to a pressure of 40 mm Hg, mimicking the diastolic pressure. Precoronary perfusate flow (CF) was measured using an electromagnetic flow meter. The change in left ventricular isovolumetric pressure amplitude (LVP) calculated by subtracting the diastolic from the systolic pressure, the rate of maximal left ventricular pressure rise (dP/dt_{max}), and the rate of the spontaneously beating heart (HR) were continuously monitored. Following at least 1 h of perfusion and stabilization of all cardiac parameters, the test drug concentration was administered via a second column and increased threefold only after the previous concentration had produced stable maximal effects. The drug-induced change in cardiac parameters was expressed as percentage of the initial values, and the responses were quantified by means of IC_{50} values (left ventricular pressure, rate of maximal left ventricular pressure rise), IC_{25} values (heart rate) and EC_{50} values (coronary flow).

2.11. Guinea pig papillary muscle: electrophysiology

Male guinea pigs (430–450 g) were killed by a blow on the head. Right ventricular papillary muscles <1 mm diameter were carefully removed from the excised hearts and maintained in oxygenated Krebs solution. The base of the muscle was clamped between a plastic block and a stainless-steel plate in a small plastic bath, perfused with Krebs solution at 10 ml/min. The tendon with attached valve was connected to an isometric force transducer (Statham UC2) and the preload was set to 0.4 g. Muscles were stimulated via a pair of platinum wires placed immediately under the tissue at 0.5 Hz, 1 ms at 5–10 V. Potentials were recorded intracellularly with glass microelectrodes filled with 3 M KCl and connected to a high impedance voltage follower (Axoclamp II). The maximum upstroke velocity of the action potential was determined with an analogue differentiator at 25 kHz. After amplification, the potentials and force of contraction were analysed online using a data acquisition system (APA II module, Notocord, France). Muscles were allowed to equilibrate for 1 h before the experiments commenced. After a successful impalement of a muscle fibre, 30 min of control data were collected. After that, aliquots of the test drug were cumulatively added to the perfusate. Each concentration was applied for 20 min or until a plateau effect was reached. All experiments were performed at 34 °C in Krebs solution of (mM) NaCl 118.0, KCl 3.4, $CaCl_2$ 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 5.5 and Na pyruvate 2.0, and gassed with 95% O_2–5% CO_2.

2.12. Calculation of antagonist affinities

From the EC_{50} values of the agonist in the presence and absence of different antagonist concentrations, concentration-ratios (designated × on the ordinate in the Schild plots) were calculated. Schild plots were constructed to estimate the pA_2 value of the antagonist and the slope of regression line (β) from each experimental series, which generally comprised at least three different concentrations (Arunlakshana and Schild, 1959). If the regression was linear and had a slope not significantly different from unity ($P>0.05$), the regression was recalculated with a constrained slope of unity (Tables 1 and 2). In those cases where β differed significantly from unity ($P<0.05$), pA_2 values determined from constrained regression lines ($\beta=1$) should be regarded as approximations. In some cases (see Tables 1 and 2), pA_2 values were calculated from the lowest single concentration of the antagonist. All data are presented as means ± S.E.M.

Competition radioligand binding experiments at α_1- and α_2-adrenoceptor subtypes were analyzed by fitting monophasic sigmoidal functions to the experimental data using the Inplot program (Graph Pad Software, San Diego, USA). The Cheng and Prusoff (1973) equation was used to calculate the − log of inhibition constant of antagonist (pK_i).

2.13. Drugs

(+)-Boldine (Aldrich, Deisenhofen, Germany) was dissolved in 0.01 N HCl and distilled water to prepare a 10^{-2} M stock solution which was further diluted with H_2O directly before the experiments. Rec 15/2739 (SB 216469; *N*-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxamide 2HCl) was a gift from Dr. R. Testa (Recordati, Milano, Italy).

L-765,314 (4-amino-2-[4-[1-benzyloxycarbonyl)-2(*S*)-[[1,1-dimethylethyl)amino]-carbonyl]piperazinyl]-6,7-dimethoxyquinazoline) was a gift from Merck (West Point, PA, USA). B8805-033 ((±)-1,3,5-trimethyl-6-[[3-[4-((2,3-dihydro-2-hydroxymethyl)-1,4-benzodioxin-5-yl)-1-piperazinyl]-propyl]amino]-2,4(1*H*,3*H*)-pyrimidinedione) (Byk Gulden, Konstanz, Germany). BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione 2HCl), MDL 73005EF (8-[2-(1,4-benzodioxan-2-ylmethylamino)-ethyl]-8-azaspiro[4.5]decane-7,9-dione HCl), buspirone HCl, UK 14.304 (brimonidine), BRL 44408 (2-[(4,5-dihydro-1*H*-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1*H*-isoindole) and ARC 239 (2-[2-(4-(2-methoxyphenyl)piperazi-1-yl)-ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione) (RBI, Cologne, Germany). [^{3}H]Prazosin (specific activity 72–82 Ci/mmol) and [^{3}H]RX 821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-5,5-dihydro-1*H*-imidazol HCl, approximately 50 Ci/mmol) were purchased from New England Nuclear (NEN, Dreieich, Germany). D-*cis*-Diltiazem HCl, DL-propranolol HCl and all other drugs were purchased from Sigma (Munich, Germany).

3. Results

3.1. α_{1A}-Adrenoceptors in rat vas deferens and perfused kidney

When (+)-boldine (3×10^{-8}–10^{-6} M) was equilibrated with rat vas deferens for 20 min, it caused parallel shifts to the right of the noradrenaline concentration–response curve, indicating competitive antagonism at α_{1A}-adrenoceptors in this tissue (Fig. 1, top). The Schild plot gave a pA_2 value of

Table 1
Functional affinities (pA_2) and potencies (− log ED_{50} mol) of (+)-boldine in comparison with α_1-adrenoceptor subtype-selective antagonists at subtype A in rat vas deferens (RVD) and rat kidney (RK), at subtype B in guinea pig spleen (GPS), mouse spleen (MS) and rabbit aorta (RabA), and at subtype D in rat aorta (RA) and rat pulmonary artery (RPA). With few exceptions (L-765,314 in GPS, MS and RabA), all pA_2 values (with slopes β of regression lines in parentheses) were calculated from constrained Schild plots ($\beta=1$) for competitive antagonism. The results are presented as means ± S.E.M. of $n=6$–7 for rat kidney and $n=12$–16 for pA_2 determinations for each drug in the different tissues

Tissue:	Subtype A		Subtype B			Subtype D	
Agonist:	RVD NA (pA_2)	RK NA (− log ED_{50} mol)	GPS NA (pA_2)	MS NA (pA_2)	RabA NA (pA_2)	RA NA (pA_2)	RPA Buspirone (pA_2)
(+)-Boldine	7.46 ± 0.06 (1.00)	9.53 ± 0.05	6.12 ± 0.05 (0.71)[a]	6.02 ± 0.08 (0.96)	5.98 ± 0.05 (0.90)	6.37 ± 0.05 (0.81)[a]	6.18 ± 0.04 (0.95)
Rec 15/2739	10.18 ± 0.06 (1.06)	11.11 ± 0.12	6.69 ± 0.07 (0.98)	6.99 ± 0.09[b] (–)	7.28 ± 0.10 (0.87)	7.83 ± 0.11 (0.74)[a]	8.07 ± 0.16 (0.93)
B8805-033	8.40 ± 0.07 (1.12)	9.82 ± 0.13	5.21 ± 0.08 (1.05)	5.34 ± 0.08 (0.89)	5.10 ± 0.04 (0.97)	5.52 ± 0.12 (0.88)	5.45 ± 0.07 (1.09)
L-765,314	6.38 ± 0.08 (0.85)	8.26 ± 0.07	7.22 ± 0.04[c] (0.72)[a]	n.t.	7.27 ± 0.09[c] (0.52)[a]	6.35 ± 0.06 (0.93)	6.44 ± 0.05 (0.94)
BMY 7378	6.67 ± 0.15 (0.93)	8.76 ± 0.19	6.55 ± 0.18 (1.02)	6.76 ± 0.07 (0.93)	6.42 ± 0.07 (0.89)	8.15 ± 0.16 (1.00)	8.00 ± 0.09 (1.10)
MDL 73005EF	5.84 ± 0.08 (0.93)	8.08 ± 0.22	5.88 ± 0.24 (0.73)[a]	6.30 ± 0.09 (0.83)	5.74 ± 0.09 (0.68)[a]	7.23 ± 0.14 (1.01)	7.32 ± 0.08 (0.86)

Most data for the reference antagonists on rat vas deferens, kidney and aorta, guinea pig and mouse spleen were taken from Eltze and Boer (1992), Eltze (1994, 1996) and Eltze et al. (1999).

n.t., not tested.

[a] Slope significantly different from unity ($P<0.05$).

[b] pA_2 value determined at the single concentration of 10^{-6} M.

[c] pA_2 value determined from unconstrained regression line at a slope as indicated.

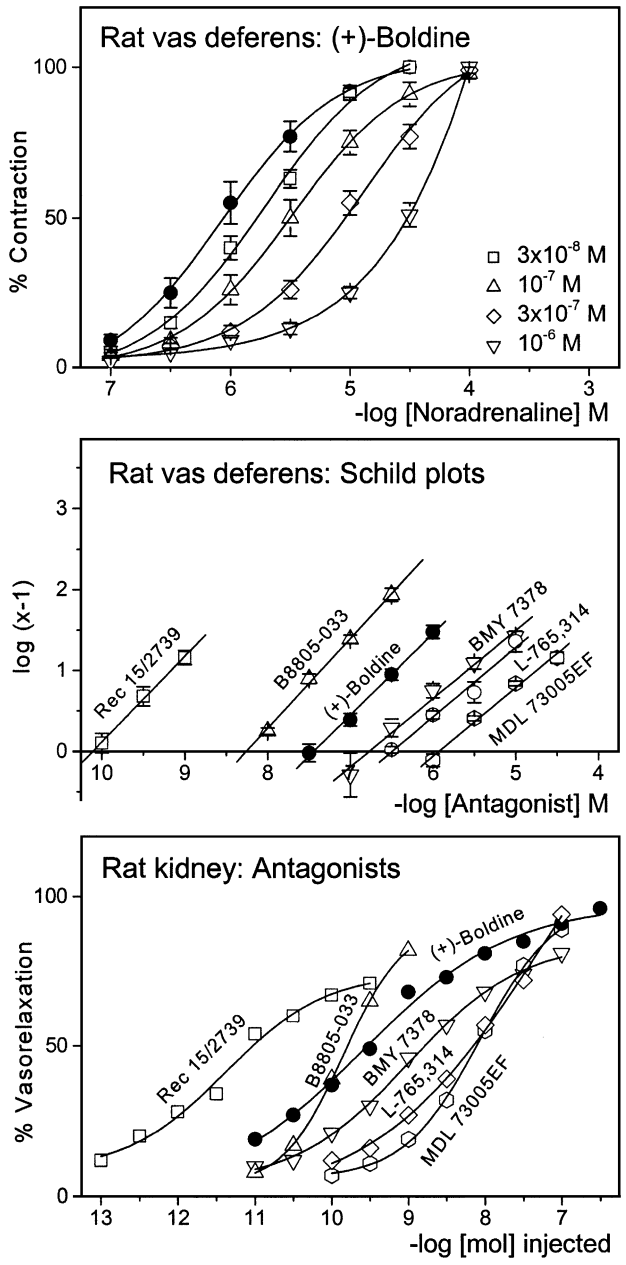

Fig. 1. Top: Concentration–response curves of noradrenaline to evoke contraction of rat vas deferens in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 20 min (means ± S.E.M., $n=12$ for the control, $n=4-9$ in the presence of each concentration of (+)-boldine). Middle: Schild plots for the antagonism by (+)-boldine and reference antagonists against noradrenaline-induced contractions in rat vas deferens (means ± S.E.M. of $n=4-9$). Bottom: Dose–response curves for the inhibition by (+)-boldine and reference antagonists of renal vasoconstriction induced by a continuous presence of 6×10^{-7} M noradrenaline in perfused rat kidney (means ± S.E.M. <5% for (+)-boldine and <10% for the reference compounds, not shown; $n=5-7$).

7.46 from the regression line of $\beta=1.00\pm0.06$ (Fig. 1, middle). The reference antagonists, Rec 15/2739, B8805-033, BMY 7378 L-765,314 and MDL 73005EF, competitively antagonized these contractions, yielding pA_2 values of 10.18, 8.40, 6.67, 6.38 and 5.84, respectively (Table 1; some data taken from Eltze et al., 2001b).

During vasoconstriction evoked in rat perfused kidney by noradrenaline (6×10^{-7} M), injections of increasing doses of (+)-boldine (10^{-11}–3×10^{-7} mol) caused a dose-dependent and reversible increase in perfusion flow (Fig. 1, bottom). The $-\log ED_{50}$ (mol) value for a half-maximal blocking effect by (+)-boldine was 9.53 (Table 1). As shown before by a series of other α_1-adrenoceptor subtype discriminating antagonists (Eltze et al., 1991; Eltze and Boer, 1992), an identical rank order resulted when the potencies of (+)-boldine and the reference antagonists to attenuate noradrenaline-induced vasoconstriction were compared with the affinity data for competitive α_{1A}-adrenoceptor antagonism in rat vas deferens, namely, Rec 15/2739>B8805-033>(+)-boldine>BMY 7378>L-765,314 ≥ MDL 73005EF (Fig. 1, middle and bottom).

3.2. α_{1A}-Adrenoceptors of α_{1L}-like state in rabbit spleen

On the rabbit spleen, (+)-boldine (3×10^{-6}–10^{-4} M) competitively antagonized the contraction to noradrenaline but was less potent, yielding a pA_2 value of 5.61 from the constrained regression line (5.67 ± 0.03 at $\beta=0.94\pm0.02$; not significantly different from 1.00, $P>0.05$) (Fig. 2, top and bottom). The affinities (pA_2 values) of some reference antagonists, prazosin, HV 723, RS-17053 and BMY 7378, on rabbit spleen were 8.27 (at $\beta=0.64$), 7.12 (at $\beta=0.90$), 6.50 (at $\beta=0.69$) and 5.81 (at $\beta=0.81$), respectively (Fig. 3, bottom; some values taken from Eltze et al., 1999), and agree well with their affinities at rabbit urethral L-like state α_{1A}-adrenoceptors (p$A_2=8.11$, 7.68, 5.86 and 5.67, respectively; Leonardi et al., 1997; Testa et al., 1997; van der Graaf et al., 1997).

3.3. α_{1B}-Adrenoceptors in guinea pig spleen, mouse spleen and rabbit aorta

In spleen from guinea pig (Fig. 3, top) and mouse (Fig. 3, middle) as well as in rabbit aorta (Fig. 3, middle), (+)-boldine at concentrations of 10^{-6}–3×10^{-5} M caused competitive and concentration-related antagonism against tissue contraction evoked by noradrenaline; however, in guinea pig spleen the shift of the agonist curve was weaker than expected for competitive antagonism. The pA_2 value calculated from a constrained Schild plot amounted to 6.12 in guinea pig spleen (p$A_2=6.47\pm0.05$ at $\beta=0.71\pm0.04$, significantly different from 1.00, $P<0.05$), 6.02 in mouse spleen (p$A_2=6.06\pm0.05$ at $\beta=0.96\pm0.06$, not significantly different from 1.00, $P>0.05$), and 5.98 in rabbit aorta (p$A_2=6.06$ at $\beta=0.90\pm0.03$, not significantly different from 1.00, $P>0.05$) (Fig. 3, bottom), the latter value being consistent with those calculated for (+)-boldine at guinea pig and mouse splenic α_{1B}-adrenoceptors. Also, the affinities determined in rabbit aorta for the B-subtype selective L-765,314 (p$A_2=7.27$), the A/B-subtype discriminating Rec

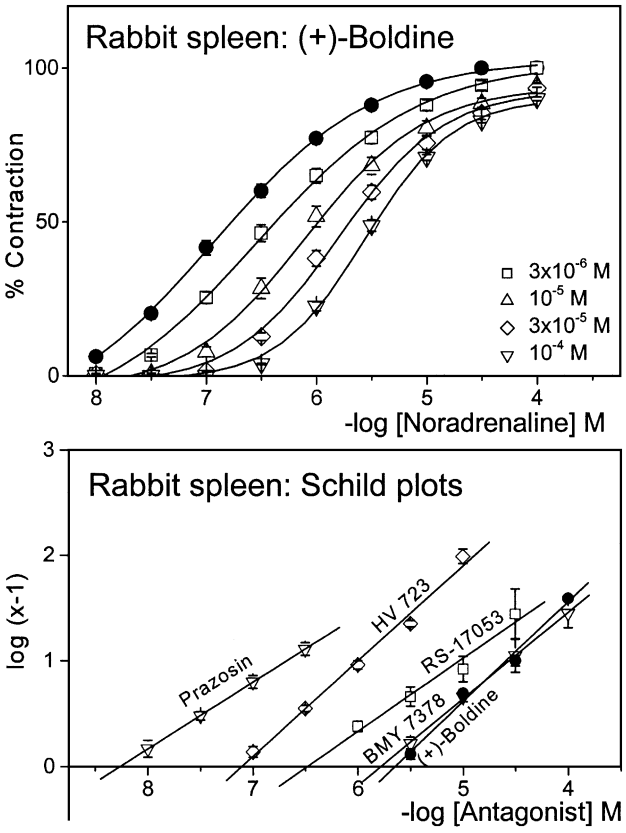

Fig. 2. Top: Concentration–response curves of noradrenaline to evoke a contraction of rabbit spleen in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n=18$ for the control, $n=9$ in the presence of each concentration of (+)-boldine). Bottom: Schild plots for the antagonism by (+)-boldine and reference antagonists against contractions evoked by noradrenaline in rabbit spleen (means ± S.E.M., $n=9-12$).

15/2739 and B8805-033 (pA_2 = 7.28 and 5.10, respectively), and the D/B-subtype discriminating BMY 7378 and MDL 73005EF (pA_2 = 6.42 and 5.74, respectively), were consistent with those at guinea pig and mouse splenic α_{1B}-adrenoceptors (Table 1).

3.4. α_{1D}-Adrenoceptors in rat thoracic aorta and pulmonary artery

In rat thoracic aorta, (+)-boldine (3×10^{-7}–3×10^{-5} M) acted as a competitive antagonist against noradrenaline-

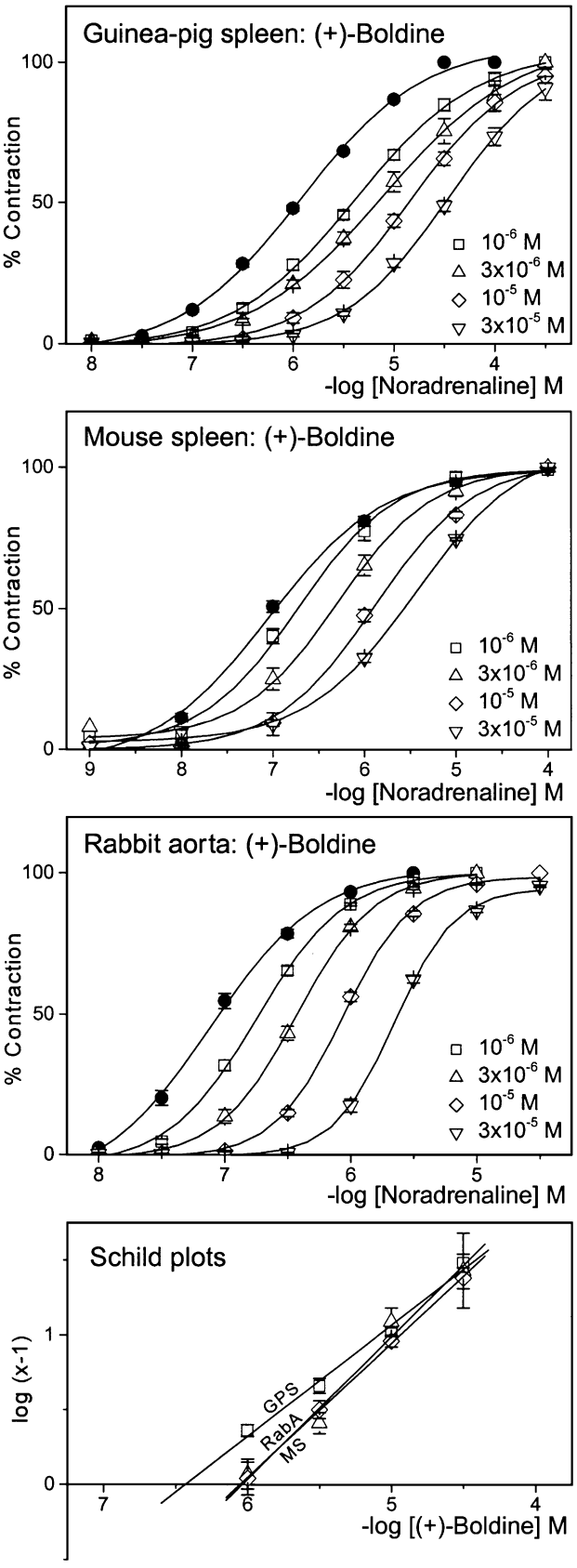

Fig. 3. Concentration–response curves of noradrenaline to evoke a contraction of guinea pig spleen (top), mouse spleen and rabbit aorta (middle) in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n=18$ for the control, $n=9$ in the presence of each concentration of (+)-boldine). Bottom: Schild plots for the antagonism by (+)-boldine against contractions evoked by noradrenaline in guinea pig spleen (GPS), mouse spleen (MS) and rabbit aorta (RabA) (means ± S.E.M., $n=9-12$).

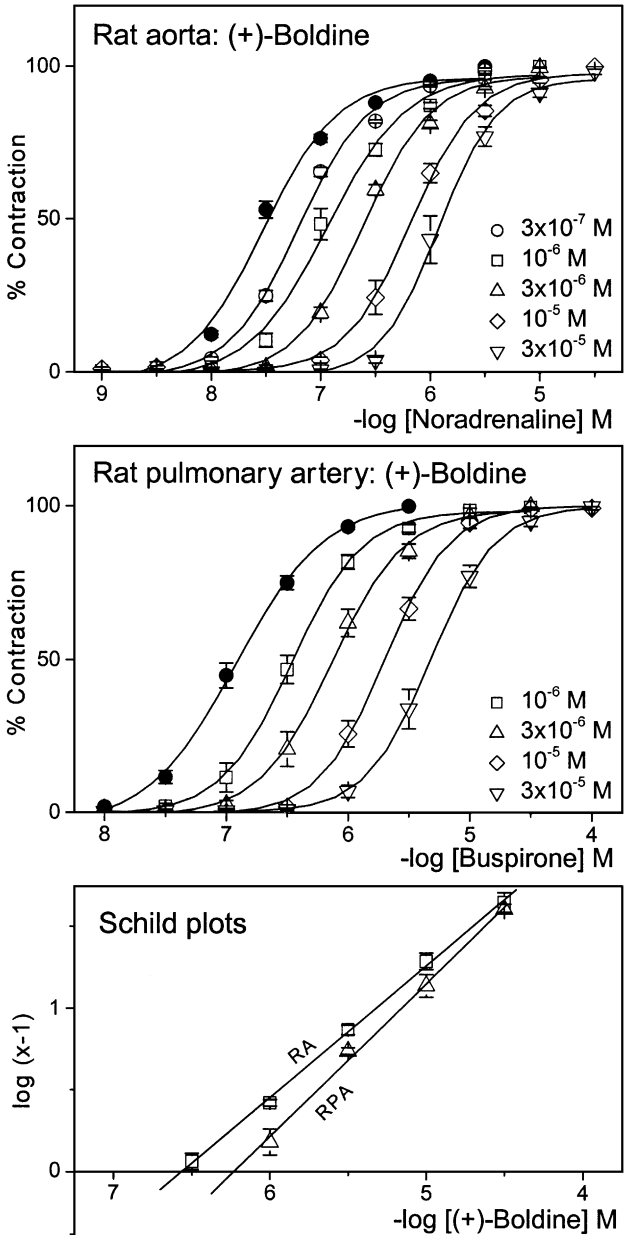

Fig. 4. Concentration–response curves of noradrenaline to evoke a contraction of rat aorta (top) and of buspirone in rat pulmonary artery (middle) in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n=18$ for the control, $n=9$ in the presence of each concentration of (+)-boldine). Bottom: Schild plots for the antagonism by (+)-boldine against contractions evoked by noradrenaline in rat aorta (RA) and against buspirone in rat pulmonary artery (RPA) (means±S.E.M., $n=9-12$).

evoked smooth muscle contraction (Fig. 4, top). A pA_2 of 6.37 (6.57 ± 0.05 at $\beta=0.81\pm0.03$, significantly different from 1.00, $P<0.05$) was obtained (Fig. 4, bottom; Table 1). When buspirone was used in rat pulmonary artery to selectively stimulate α_{1D}-adrenoceptors in this tissue (Eltze et al., 1999), (+)-boldine between 10^{-6} and 3×10^{-5} M caused parallel shifts to the right of the agonist curve (Fig. 4, middle). In the constrained Schild plot regression, a pA_2 value of 6.18 (6.23 ± 0.04 at $\beta=0.95\pm0.03$, not significantly different from 1.00, $P>0.05$) was calculated (Fig. 4, bottom), which corresponds to that obtained in rat aorta against noradrenaline (Table 1).

3.5. Cloned human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in CHO cells

(+)-Boldine competed for the cloned human α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors stably expressed in CHO cells with pK_i values of 7.21, 5.79 and 6.09, respectively (Fig. 5, top; Table 3), and displayed an affinity rank order at these binding sites, namely, $\alpha_{1A}>\alpha_{1B}=\alpha_{1D}$, identical to that obtained from functional studies (Table 1). Its selectivity was 26- and 13-fold for α_{1A}- vs. α_{1B}- and α_{1D}-adrenoceptors, respectively.

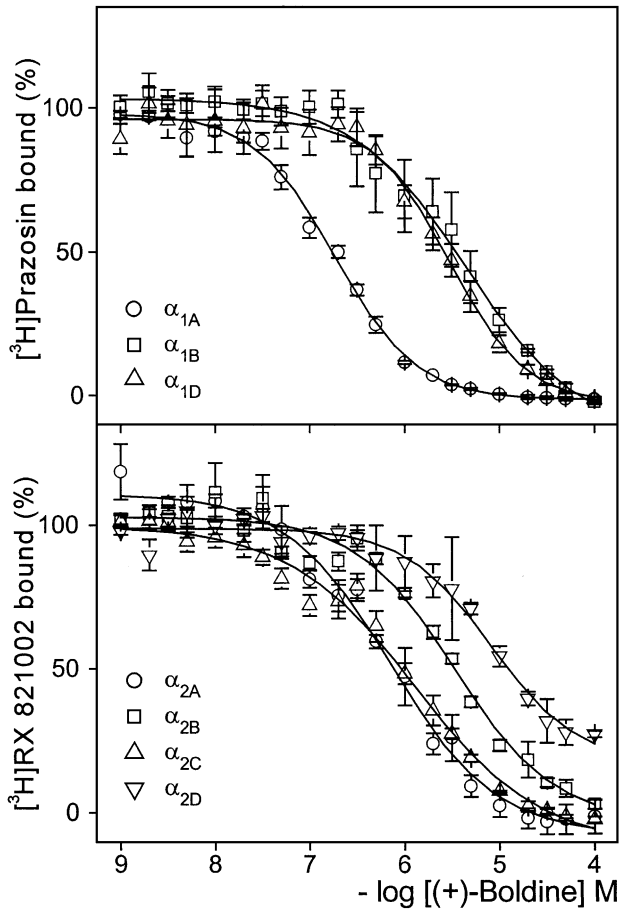

Fig. 5. Top: Radioligand displacement curves depicting the competition between (+)-boldine and [^{3}H]prazosin at human cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors stably expressed in CHO cells (means ± S.E.M. of $n=4$ experiments). Bottom: Radioligand displacement curves depicting the competition between (+)-boldine and [^{3}H]RX 821002 at human cloned α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors stably expressed in HEK cells, and at rat cortical α_{2D}-adrenoceptors (means ± S.E.M. of $n=4$ experiments).

3.6. Prejunctional α_2-adrenoceptors in field-stimulated rat and rabbit vas deferens

The antagonism by (+)-boldine on prejunctional α_2-adrenoceptors was studied in the field-stimulated rat and rabbit vas deferens. In these tissues, the concentration–response curves for UK 14.304 (rat vas deferens: $EC_{50} = 4.15 \pm 0.07 \times 10^{-9}$ M, i.a. = 0.80 ± 0.05; rabbit vas deferens: $EC_{50} = 1.31 \pm 0.05 \times 10^{-9}$ M, i.a. = 0.84 ± 0.06; means ± S.E.M., $n = 22–33$) were shifted to the right in a concentration-dependent manner by (+)-boldine (10^{-6}–3×10^{-5} M in rat vas deferens, 3×10^{-7}–10^{-5} M in rabbit vas deferens). In rat vas deferens, these curves were clearly consistent with competitive antagonism, but not fully in rabbit vas deferens (Figs. 6 and 7, top). The pA_2 values were 6.02 (5.99 ± 0.03 at $\beta = 1.04 \pm 0.12$, not significantly different from 1.00, $P > 0.05$) in rat vas deferens, and 6.36 (6.29 ± 0.05 at $\beta = 1.12 \pm 0.10$, not significantly different from 1.00, $P > 0.05$) in rabbit vas deferens (Figs. 6 and 7, bottom; Table 2). As reference standards, we found ARC 239 to be a relatively weak competitive antagonist with a pA_2 of 5.68 in rat vas deferens (pA_2 = 5.74 ± 0.18 at $\beta = 0.84 \pm 0.16$, not significantly different from 1.00, $P > 1.00$) (Fig. 6, middle and bottom; Table 2), but ARC 239 failed to affect the inhibitory action of UK 14.304 on twitch contractions in rabbit vas deferens up to 3×10^{-5} M (not shown). In the presence of BRL 44408 (3×10^{-8}–3×10^{-7} M), concentration–response curves to UK 14.304 in rabbit vas deferens were competitively shifted to the right, resulting in a pA_2 value of 7.41 (7.40 ± 0.05 at $\beta = 1.02 \pm 0.06$, not significantly different from 1.00, $P > 0.05$) (Fig. 7, middle and bottom, Table 2). The respective affinity for BRL 44408 (pA_2 = 7.63) at rat vas deferens α_2-adrenoceptors was taken from Smith et al. (1992). Also, the pA_2 values for yohimbine (7.69 in rat vas deferens, 7.33 in rabbit vas deferens) were nearly identical (Table 2).

3.7. Prejunctional α_2-adrenoceptors in sympathetic-nerve stimulated rat left atrium

UK 14.304 (10^{-9}–3×10^{-7} M) was found to be an effective inhibitor of neurotransmission in the rat left atrium, producing a concentration-dependent and maximally 90% inhibition of the inotropic response to brief, intermittent sympathetic nerve stimulation ($EC_{50} = 7.4 \times 10^{-9}$ M). Fig. 8 shows that (+)-boldine (3×10^{-6}–3×10^{-5} M) caused a rightward shift of the agonist curve; however, the shift was weaker than expected for competitive antagonism. The pA_2 value calculated from a constrained Schild plot including all three concentrations of (+)-boldine amounted to 5.89 (6.34 ± 0.24 at $\beta = 0.66 \pm 0.08$, significantly different from 1.00, $P > 0.05$) (Fig. 8, bottom), but was higher (pA_2 = 6.06 ± 0.24) using the lowest single concentration of 3×10^{-6} M; this value is included in Table 2. Respective potencies for BRL 44408 and ARC 239 ($-\log EC_{30}$ = 6.49 and 7.40, respectively), and affinity for yohimbine (pA_2 = 7.77) at rat left atrial α_{2B}-adrenoceptors were taken from Smith et al. (1992) and Alabaster et al. (1986) (Table 2).

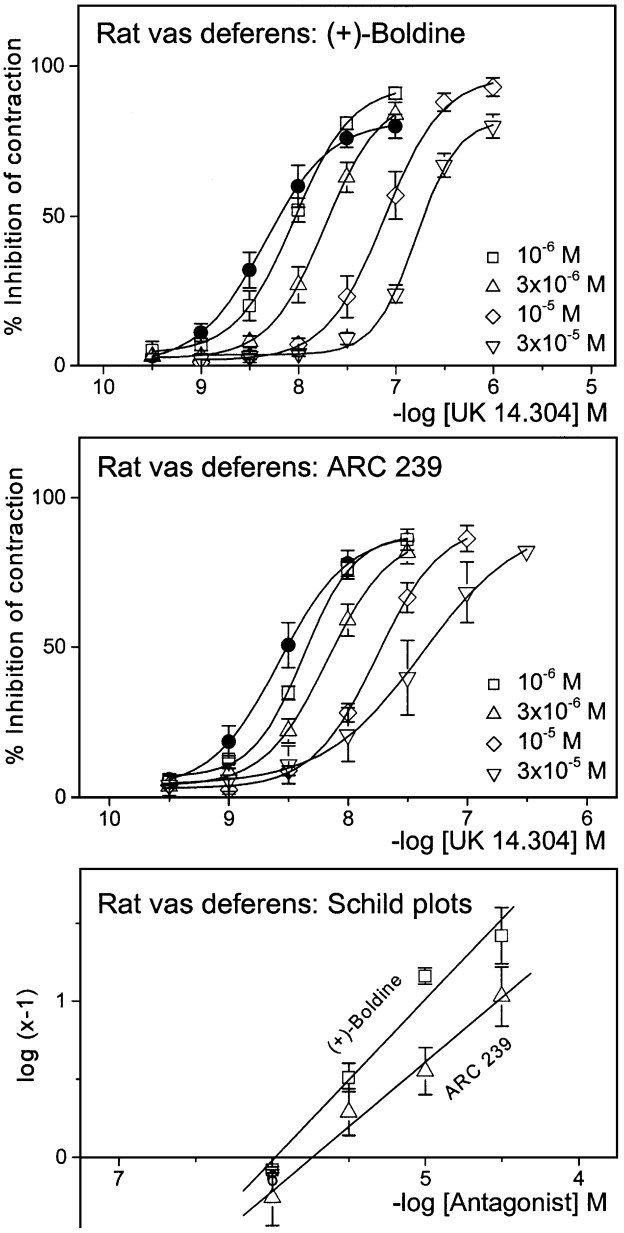

Fig. 6. Effect of UK 14.304 on twitch contractions evoked by electrical field stimulation of rat vas deferens in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols, top) and ARC 239 (open symbols, middle) equilibrated with the tissue for 30 min (means ± S.E.M., $n = 18$ for the control, $n = 9$ in the presence of each concentration of the antagonists). Bottom: Schild plots for the antagonism by (+)-boldine and ARC 239 against the UK 14.304-induced inhibition of twitch contractions in rat vas deferens (means ± S.E.M. , $n = 4–5$).

3.8. Prejunctional α_{2D}-adrenoceptors in field-stimulated guinea pig ileum

In the field-stimulated longitudinal muscle strip of the guinea pig ileum, UK 14.304 caused an inhibition of twitch

Table 2
Functional affinities of (+)-boldine in comparison with the $\alpha_{2A/D}$-selective BRL 44408, the α_{2B}-selective ARC 239 and the unselective yohimbine at prejunctional α_2-adrenoceptors in different tissues. All pA_2 values (with slopes β of regression lines in parentheses), except those for (+)-boldine in rat left atrium and guinea pig ileum and for ARC 239 in guinea pig ileum, were calculated from constrained Schild plots ($\beta = 1$) for competitive antagonism. The results are presented as means ± S.E.M. of $n = 7–9$ for pA_2 determinations for each drug in the different tissues

Tissue	Rat vas deferens	Rabbit vas deferens	Rat left atrium	Guinea pig ileum
(+)-Boldine	6.02 ± 0.03 (1.04 ± 0.12)	6.36 ± 0.05 (1.12 ± 0.10)	6.06 ± 0.24[a]	4.38 ± 0.26[b]
BRL 44408	7.63[1]	7.41 ± 0.05 (1.02)	6.49[1]	8.05 ± 0.08 (1.17)
ARC 239	5.68 ± 0.18 (0.84)[c]	< 4.50	7.40[1]	5.64 ± 0.09[a]
Yohimbine	7.69 ± 0.03 (0.89)	7.33 ± 0.10 (1.00)	7.77[2]	7.13 ± 0.17 (1.13)

Some values determined under similar conditions were taken from [1]Smith et al. (1992) (pA_2 value in rat vas deferens; $-\log(EC_{30})$ M for a 30% increase in stimulation-evoked overflow of tritium by antagonist in rat left atrium) and [2]Alabaster et al. (1986).
[a] pA_2 value determined at the single concentration of 3×10^{-6} M.
[b] pA_2 value determined at the single concentration of 3×10^{-5} M.
[c] Slope significantly different from unity ($P < 0.05$).

contractions ($EC_{50} = 5.01 \pm 0.18 \times 10^{-8}$ M; i.a. = 0.66 ± 0.08; means ± S.E.M., $n = 24$). (+)-Boldine at concentrations between 10^{-6} and 10^{-5} M was inactive against UK 14.303, but at 3×10^{-5} and 10^{-4} M caused a concentration-dependent rightward shift of the agonist curve; however, at 10^{-4} M the shift of the agonist curve was greater than expected for competitive antagonism (p$A_2 = 4.46 \pm 0.04$ at $\beta = 2.27 \pm 0.27$, significantly different from 1.00, $P < 0.01$) (Fig. 8, middle and bottom). Using the lowest single concentration of 3×10^{-5} M, a pA_2 value of 4.38 ± 0.26 was calculated (Table 2). However, during equilibration with the stimulated guinea pig ileum, (+)-boldine at 10^{-5}, 3×10^{-5} and 10^{-4} M, caused an 8 ± 2%, 26 ± 4% and 45 ± 4% inhibition of twitch contraction, respectively (means ± S.E.M., $n = 8–12$), possibly mediated through an anticholinergic action previously observed also on rat ileum (Speisky et al., 1991b) or by its known Ca^{2+} channel antagonism (Ivorra et al., 1993a,b), rendering exact pA_2 calculation of (+)-boldine at guinea pig ileum α_{2D}-adrenoceptors difficult. A low affinity value was also found for ARC 239 in the field-stimulated guinea pig ileum (p$A_2 = 5.58$ calculated at the single concentration of 3×10^{-6} M, above which the drug was incompletely dissolved in the bathing solution and caused no further shift), while the values for yohimbine ($10^{-7}–10^{-6}$ M) and BRL 44408 ($3 \times 10^{-8}–3 \times 10^{-7}$ M) amounted to 7.13 (7.06 ± 0.17 at $\beta = 1.13 \pm 0.19$, not significantly different from 1.00, $P > 0.05$) and 8.05 (7.90 ± 0.08 at β = 1.17 ± 0.11, not significantly different from 1.00, $P > 0.05$), respectively (Table 2).

3.9. Cloned human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors in HEK cells and rat cortical α_{2D}-adrenoceptors

Using the membranes prepared from HEK cells expressing the subtypes of human α_2-adrenoceptor subtypes A, B and C, (+)-boldine displaced the binding of [^{3}H]RX 821002 in a specific, concentration-dependent and complete manner resulting in pK_i values of 6.26, 5.79 and 6.35, respectively. The affinity of (+)-boldine at rat cortical α_{2D}-adrenoceptors was much lower (p$K_i = 4.70$) (Fig. 5, bottom; Table 3). A similar affinity rank order to the three α_2-adrenoceptors (i.e. A ≥ B>D) was found in binding and functional studies (Table 2 and 3). In binding studies, (+)-boldine's selectivity was 3- and 36-fold for α_{2A}- vs. α_{2B}- and α_{2D}-adrenoceptors, respectively.

3.10. Inhibition of K^+-evoked vasoconstriction in perfused rat kidney

During vasoconstriction in rat kidney evoked by permanent perfusion of the organ with 27 mM K^+, injections of increasing doses of (+)-boldine ($10^{-8}–5 \times 10^{-6}$ mol) or diltiazem ($10^{-10}–3 \times 10^{-6}$ mol) caused a dose-dependent and reversible dilatation (Fig. 9). As deduced from their ability to cause a 50% inhibition of vasoconstriction ($-\log ED_{50}$ mol: 6.03 ± 0.09 for (+)-boldine vs. 7.89 ± 0.03 for diltiazem; means ± S.E.M., $n = 5–6$), (+)-boldine proved to be 70-fold less potent than diltiazem.

3.11. Cardiac effects in guinea pig Langendorff heart

The average initial values of cardiac parameters measured in guinea pig perfused Langendorff hearts were as follows: coronary flow (CF) = 11.4 ± 0.8 ml min^{-1}; left ventricular pressure amplitude (LVP) = 76 ± 6 mm Hg; rate of maximal left ventricular pressure rise (dP/dt_{max}) = 1810 ± 109 mm Hg s^{-1}; and the rate of the spontaneously beating heart (HR) = 179 ± 6 min^{-1} (means ± S.E.M., $n = 10$). There were no significant differences ($P > 0.05$) between the initial values of the two series of experiments.

(+)-Boldine (between 10^{-5} and 2×10^{-4} M) produced a concentration-dependent increase in coronary flow accompanied by a decrease in left ventricular pressure and rate of maximal left ventricular pressure rise (Fig. 10, top). From the nearly identical concentrations of (+)-boldine necessary to evoke a 50% increase in coronary flow and a decrease in left ventricular pressure of the same amount ($EC_{50} = 3.7 \times 10^{-5}$ M vs. $IC_{50} = 5.7 \times 10^{-5}$ M), no vascular selectivity could be detected for the compound, while at this concentrations heart rate was also reduced between 35% and 50%. Surprisingly, at much lower concentrations

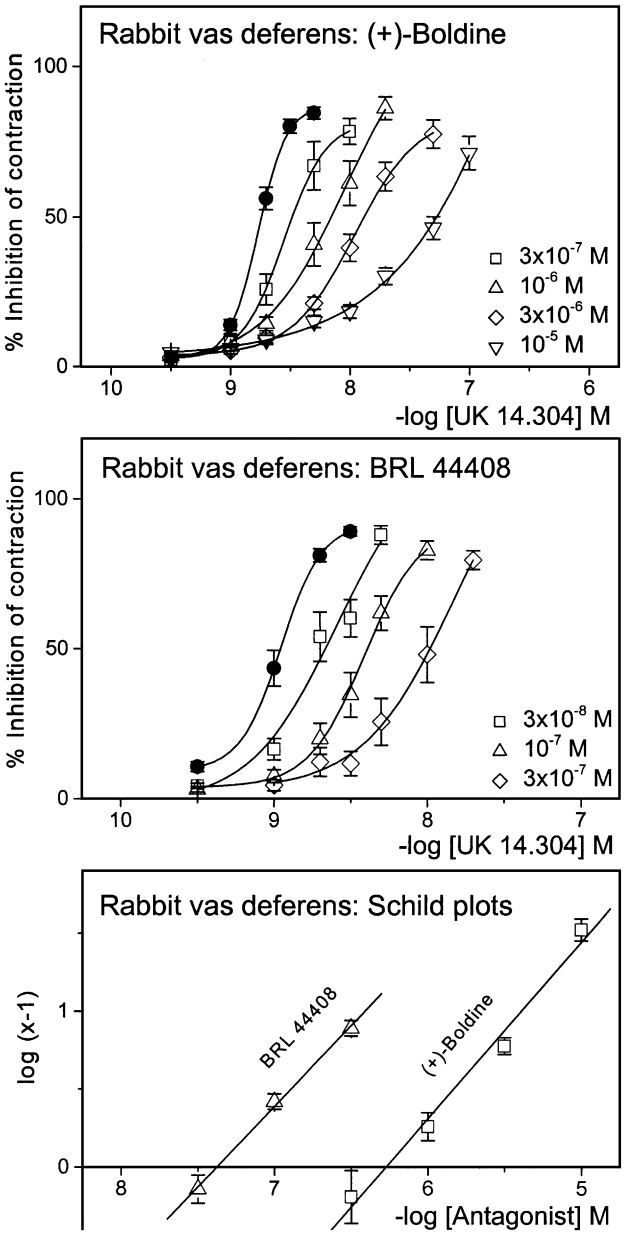

Fig. 7. Effect of UK 14.304 on twitch contractions evoked by electrical field stimulation of rabbit vas deferens in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols, top) and BRL 44408 (open symbols, middle) equilibrated with the tissue for 30 min (means ± S.E.M., $n=18$ for the control, $n=8-9$ in the presence of each concentration of the antagonists). Bottom: Schild plots for the antagonism by (+)-boldine and BRL 44408 against the UK 14.304-induced inhibition of twitch contractions in rabbit vas deferens (means ± S.E.M., $n=9-12$).

(3×10^{-7}–10^{-5} M) than those causing vasodilation, (+)-boldine already decreased heart rate between 16% and 22% ($IC_{25}=3 \times 10^{-5}$ M). Diltiazem (between 10^{-7} and 10^{-5} M) proved to be approximately 50-fold more potent in terms of increasing coronary flow ($EC_{50}=7 \times 10^{-7}$ M) and depressing left ventricular pressure ($IC_{50}=9 \times 10^{-7}$ M) or rate of maximal left ventricular pressure rise; however, compared to (+)-boldine, its negative chronotropic action at these concentration was weaker (−7% to −14%; $IC_{25}=2.5 \times 10^{-6}$ M) (Fig. 10, bottom). Thus, a difference in potency to cause a negative inotropic related to a negative

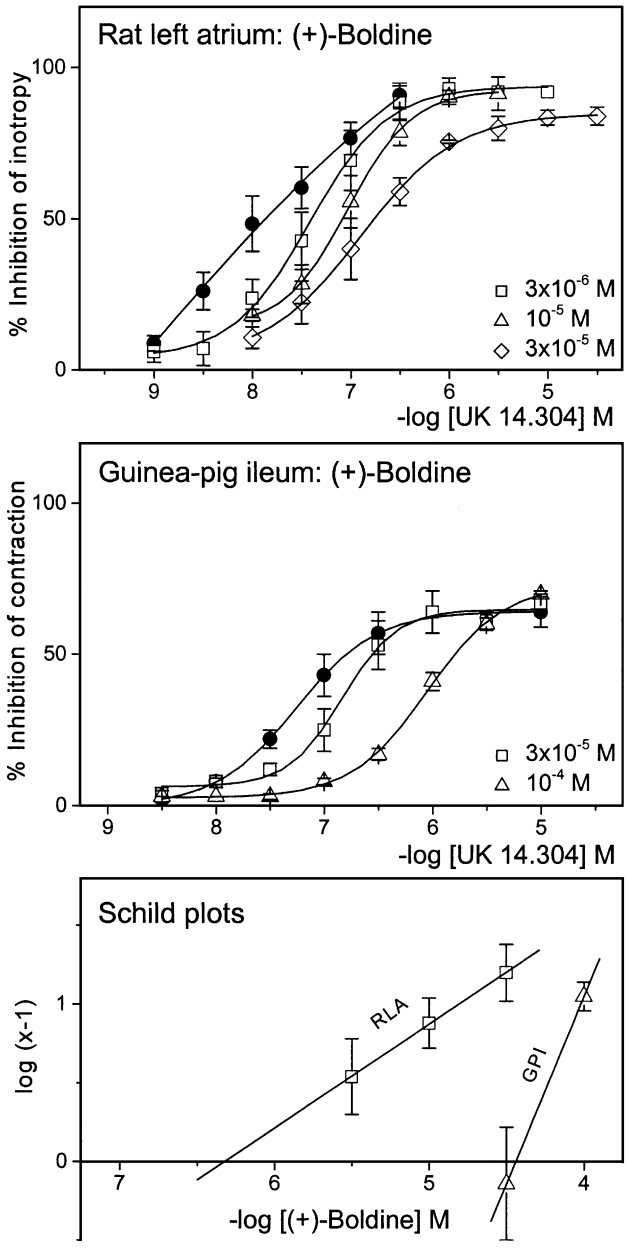

Fig. 8. Top: Effect of UK 14.304 on the response of the rat left atrium to sympathetic nerve stimulation in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n=9$ for the control, $n=3$ in the presence of each concentration of the antagonist). Middle: Effect of UK 14.304 on twitch contractions evoked by electrical field stimulation of guinea pig ileum in the absence (filled circles) or presence of increasing concentrations of (+)-boldine (open symbols) equilibrated with the tissue for 30 min (means ± S.E.M., $n=18$ for the control, $n=9$ in the presence of each concentration of the antagonist). Bottom: Schild plots for the antagonism by (+)-boldine against the UK 14.304-induced inhibition of inotropic response to sympathetic nerve stimulation in rat left atrium (RLA), and of twitch contractions in guinea pig ileum (GPI) (means± S.E.M., $n=3-9$).

Table 3
Binding affinities of (+)-boldine at cloned human α_1-adrenoceptors (subtypes A, B and D) stably expressed in Chinese hamster ovary (CHO) cells, at α_2-adrenoceptors (subtypes A, B and C) stably expressed in human embryonic kidney (HEK) cells, and at rat cortical α_{2D}-adrenoceptors. Results are given as means ± S.E.M. of $n=4$

Subtype	pK_i
α_1-Adrenoceptor subtypes	
Human α_{1A}	7.21 ± 0.05
Human α_{1B}	5.79 ± 0.10
Human α_{1D}	6.09 ± 0.08
α_2-Adrenoceptor subtypes	
Human α_{2A}	6.26 ± 0.09
Human α_{2B}	5.79 ± 0.03
Human α_{2C}	6.35 ± 0.10
Rat α_{2D}	4.70 ± 0.29

chronotropic effect could be observed for both drugs, as expressed by their inotropic–chronotropic ratios (IC_{50} inotropy/IC_{25} chronotropy) of 1.9 for (+)-boldine, but 0.36 for diltiazem. Additionally, a greater maximal decrease in heart rate was observed for (+)-boldine (− 96 ± 2%) compared to that elicited by diltiazem (− 34 ± 14%; mean ± S.E.M., $n=4–6$). In respect to evoke coronary dilation and to depress left ventricular pressure, (+)-boldine proved to be 53- and 63-fold less potent, respectively, than diltiazem.

3.12. Electrophysiology in guinea pig papillary muscle

In guinea pig papillary muscle stimulated at 0.5 Hz and perfused with increasing concentrations of (+)-boldine ($10^{-7}–10^{-4}$ M), each concentration being applied for 30 min, the predominant effects occurring at concentrations between 10^{-6} and 10^{-5} M were to prolong the action potential duration (APD) at higher levels of repolarization (>30%), and to slightly increase the papillary force of

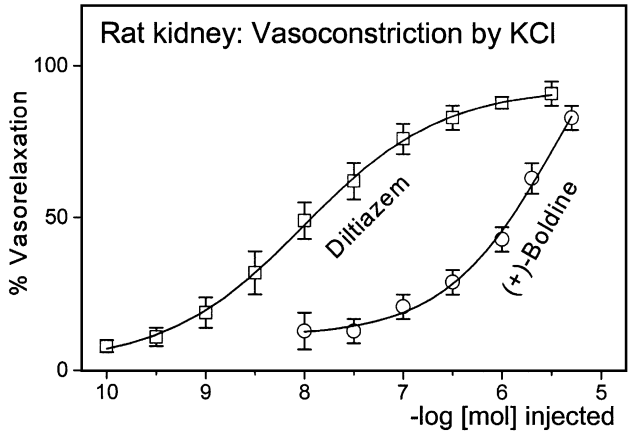

Fig. 9. Dose–response curves for the inhibition by (+)-boldine and diltiazem of renal vasoconstriction induced by continuous perfusion of 27 mM KCl in perfused rat kidneys (means ± S.E.M., $n=5–6$).

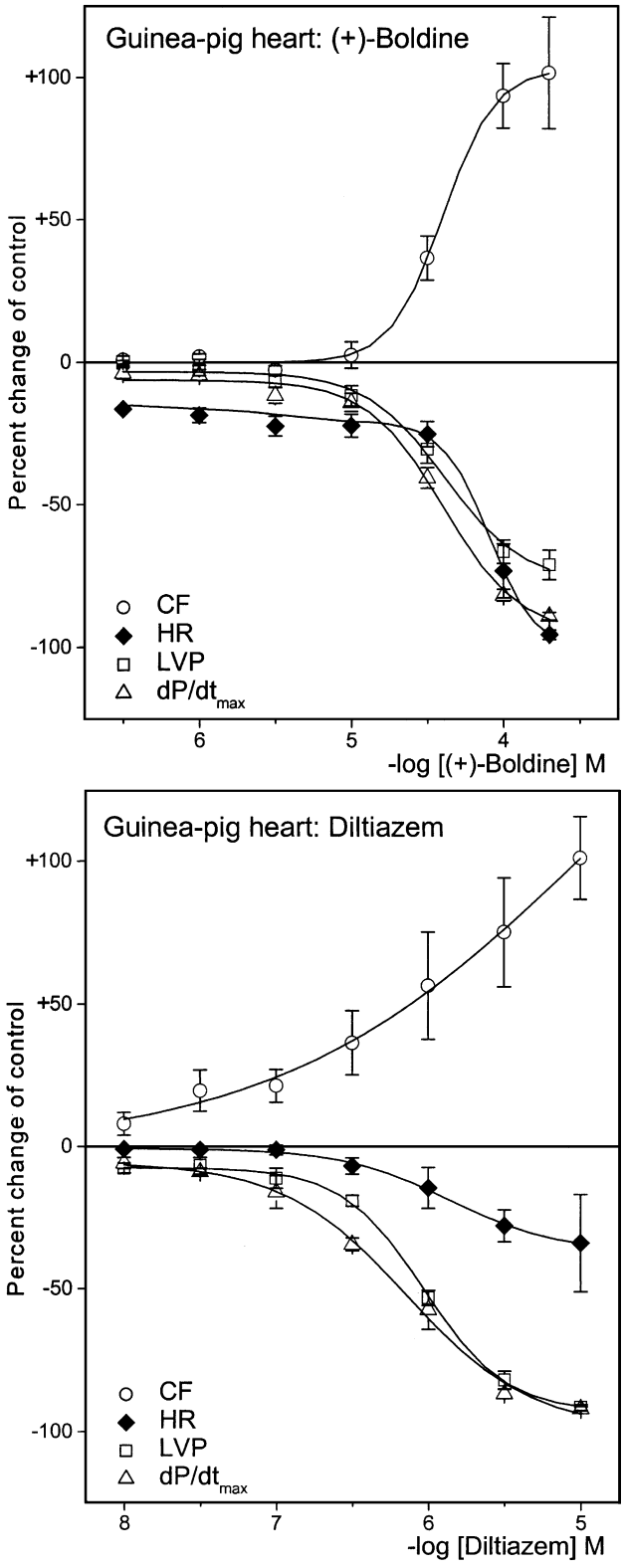

Fig. 10. Effects of (+)-boldine (top) in comparison with diltiazem (bottom) on coronary flow (CF), heart rate (HR), left ventricular pressure (LVP) and its rate of maximal left ventricular pressure rise (dP/dt_{max}) in perfused Langendorff hearts of guinea pigs. Points show the percent change in response to drug perfusion related to pre-drug values (means ± S.E.M., $n=4–6$).

contraction (up to 15%), the latter being converted to negative inotropy after 10^{-4} M of (+)-boldine. It was consistently found that the maximum increase of APD_{50} and APD_{90} occurred at the concentrations of 10^{-5} and 10^{-4} M, respectively, with no significant enhancement of APD_{30}. Resting membrane potential, action potential amplitude and the maximal rate of depolarization (V_{max}) remained unaffected by (+)-boldine at concentrations between 10^{-7} and 10^{-5} M. Fig. 11 shows the typical steady-state effects of (+)-boldine (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) on action potential and force of contraction.

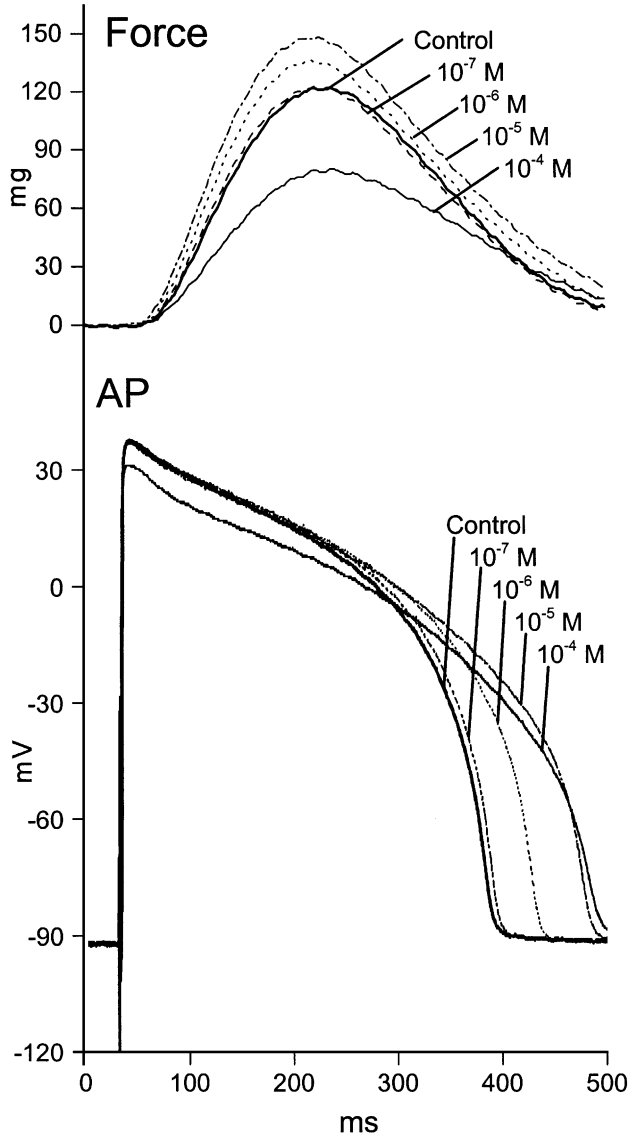

Fig. 11. Representative superimposed recordings of force of contraction (top) and action potentials (bottom) in a single superfused guinea pig right papillary muscle during control condition and after the administration of increasing concentrations of (+)-boldine (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M), each being equilibrated for 30 min. The horizontal axis shows the time in milliseconds; the vertical axis is calibrated in milligrams for the developed force of contraction and in millivolts for the action potential.

4. Discussion

4.1. General considerations

(+)-Boldine, an alkaloid isolated from the leaves and bark of the Chilean tree *Peumus boldus* traditionally employed in folk medicine and recognized as a herbal remedy, is included in a number of pharmacopoeias in South America and Europe. Within the last 10 years, there has been an increasing interest in an explanation of the mechanism(s) of action responsible for the drug's claimed plenitude of indications, comprising digestive and/or hepatobiliary disorders, headache, earache, nasal congestion, rheumatism and menstrual pain. It is also suggested to be a sedative and mild hypnotic (see Speisky and Cassels, 1994). Apart from its predominant antioxidative and cytoprotective properties (Speisky et al., 1991a; Cederbaum et al., 1992; Bannach et al., 1996), a neuroleptic-like action possibly due to its affinity at dopamine receptors (Zetler, 1988; Asencio et al., 1999), (+)-boldine has been shown to block α_1-adrenoceptors and Ca^{2+} channels (Ivorra et al., 1993a,b). In radioligand binding studies in rat cortical α_1-adrenoceptors, the drug has a 65-fold higher affinity at the native subtype A ($pK_i = 8.31$) over the subtype B ($pK_i = 6.50$) (Ivorra et al., 1995; Madrero et al., 1996). Its vasorelaxant and hypotensive activity has been ascribed to both α_1-adrenoceptor antagonism and Ca^{2+} entry blockade, the latter effect, although being up to 120-fold weaker, did characteristically occur at the diltiazem sensitive benzothiazepine binding site of the Ca^{2+} channel (Ivorra et al.,1993a; Chulia et al., 1996; Orallo et al., 1998; Fabeiro et al., 2000). In the present study, our purpose was (1) to replicate the findings for the presumed α_{1A}-adrenoceptor selectivity of (+)-boldine by using functional and radioligand binding experiments; (2) to determine its functional affinity at different α_2-adrenoceptor subtypes located at adrenergic and cholinergic nerve endings, as well as at cloned human subtypes A, B and C, as well as at subtype D in rat tissue; (3) to evaluate the strength of its Ca^{2+} channel antagonism on the vasculature in rat kidney, and on cardiac tissue in perfused guinea pig Langendorff heart with respect to coronary dilatation and cardiac depression; and 4) to investigate its effect on action potential in guinea pig papillary muscle.

4.2. Affinity at α_1-adrenoceptor subtypes

In most α_1-adrenoceptor containing tissues used, the antagonism exerted by (+)-boldine was competitive in nature, thus enabling an exact calculation of functional affinities (pA_2 values) at α_{1A}-adrenoceptors in rat vas deferens (7.46), α_{1B}-adrenoceptors in mouse spleen (6.02) and rabbit aorta (5.98), and at α_{1D}-adrenoceptors in rat pulmonary artery (6.18), whereas those determined in guinea pig spleen α_{1B}-adrenoceptors (6.12) and in rat aortic α_{1D}-adrenoceptors (6.37), due to the flat regression lines in

the Schild plots, must be regarded as approximations. An identical rank order of affinities in rat vas deferens and potencies for attenuating vasoconstriction to noradrenaline in rat kidney were obtained for (+)-boldine and the reference antagonists, making both tissues a reliable functional assay material for investigating the α_{1A} subtype (Eltze et al., 1991). Consistent pA_2 values for (+)-boldine and the α_1-adrenoceptor subtype A/B-discriminating antagonists, Rec 15/2739 and B8805-033, the subtype D/B-discriminating antagonists, BMY 7378 and MDL 73005EF, and the moderately α_{1B}-adrenoceptor selective antagonist, L-765,314, were found between rabbit aortic and both guinea pig and mouse splenic α_{1B}-adrenoceptors, confirming the previous proposal for α_{1B}-adrenoceptor stimulation in the rabbit aorta, when noradrenaline is used as the agonist to evoke contraction (Muramatsu et al., 1998; Eltze et al., 2001b). In these tissues, L-765,314 proved to be sevenfold selective for α_1-adrenoceptor subtype B over both A and D, a ratio being smaller than that (factor ≥ 40) initially determined in functional studies (Chang et al., 1998). Consistent pA_2 values for (+)-boldine and the reference antagonists were also obtained by using noradrenaline in rat aorta and buspirone in rat pulmonary artery, revealing, that by the use of the agonist buspirone in the latter tissue, a single α_1-adrenoceptor, namely, the D subtype, is activated (Eltze et al., 1999).

Additionally, we characterized (+)-boldine in radioligand binding studies at cloned human α_1-adrenoceptor subtypes A, B and D stably expressed in CHO cells (Keffel et al., 2000). The affinity data obtained thereof (pK_i at A = 7.21, B = 5.79 and D = 6.09) demonstrate a 26- and 13-fold selectivity of the compound for human α_{1A}- vs. α_{1B}- and α_{1D}-adrenoceptors, respectively, exactly corresponding to the values obtained from our functional experiments in non-human tissues.

At rabbit splenic α_{1L}-adrenoceptors (Oriowo, 1998), (+)-boldine was less potent yielding a pA_2 value of 5.63, which is consistent with that obtained in guinea pig aortic α_1-adrenoceptors (pA_2 = 5.64; Chulia et al., 1996), a tissue recently being assigned to the L-like state of subtype A (Yamamoto and Koike, 1999), after it had been controversially discussed to contain either α_{1A}- (Oriowo, 1994) or α_{1B}-adrenoceptors (Chess-Williams et al., 1996).

4.3. Affinity at α_2-adrenoceptor subtypes

(+)-Boldine shares the property of the alkaloid yohimbine to interact with α_2-adrenoceptors (Fabeiro et al., 2000). At prejunctional α_2-adrenoceptors on sympathetic nerve endings in rat vas deferens which resemble the α_{2A}-adrenoceptor binding site of human platelets (Connaughton and Docherty, 1990; Smith et al., 1992), but being different from those present in rabbit vas deferens (Lattimer and Rhodes, 1985; Alabaster et al., 1986), (+)-boldine displayed an affinity (pA_2 = 6.02) consistent with that previously found in rat vas deferens against the α_2-adrenoceptor agonist BHT-920 (pA_2 = 6.0) (Fabeiro et al., 2000). The value obtained in rabbit vas deferens (pA_2 = 6.36) was of similar magnitude. No substantial differences in affinities were observed for the $\alpha_{2A/D}$-adrenoceptor selective BRL 44408 (pA_2 = 7.63 and 7.41) and the unselective yohimbine (7.69 and 7.33) in vas deferens from rat and rabbit, respectively, whereas values for the α_{2B}-adrenoceptor selective ARC 239 expectedly were low, but different from each other (5.68 and <4.50).

The prejunctional α_2-adrenoceptor modulating the release of acetylcholine in guinea pig ileum and mediating the inhibition of cholinergic twitch contractions evoked by electrical field stimulation has been assigned to the functional subtype D (Funk et al., 1995; Colucci et al., 1998). In this tissue, (+)-boldine was a less potent antagonist (pA_2 = 4.38). The affinities for ARC 239, yohimbine and BRL 44408 (5.58, 7.13 and 8.05, respectively) found in the present study at guinea pig ileum are closely correlated with the pK_B values of the antagonists at α_{2D}-adrenoceptors in rat ileum circular muscle (5.75, 7.35 and 7.89, respectively; Liu and Coupar, 1997), and in rat submandibular gland (5.54, 7.34 and 7.77, respectively; Gavin and Docherty, 1996), thereby confirming the presence of presynaptic α_{2D}-adrenoceptors in guinea pig ileum (Funk et al., 1995; Colucci et al., 1998). Although BRL 44408 and ARC 239 have been shown to have selective actions at $\alpha_{2A/D}$- and α_{2B}-adrenoceptors, respectively, they do not greatly differentiate between α_{2A}- and α_{2D}-adrenoceptors, at which these antagonists have either high (BRL 44408, pK_i>7.5) or low (ARC 239, pK_i < 6.6) affinities (Bylund et al., 1994; Trendelenburg et al., 1997). In this respect, it is interesting to note that (+)-boldine discriminates these subtypes, displaying an approximately 40-fold lower affinity at α_{2D}-adrenoceptors in rat cortex (pK_i = 4.70) or in guinea pig ileum (pA_2 = 4.38) than at cloned human α_{2A}-adrenoceptors (pK_i = 6.26) or those located at adrenergic nerve endings in rat vas deferens (pA_2 = 6.02). It is also worth mentioning that this profile ($\alpha_{2A}>\alpha_{2D}$) is similar to that of the alkaloids yohimbine, rauwolscine and raubasine (Ruffolo et al., 1995). The pressor response to the stimulation of α_2-adrenoceptors in the pithed rat is mediated primarily by the α_{2D}-adrenoceptor (Gavin and Docherty, 1996). Hence, the low affinity of (+)-boldine for α_{2D}-adrenoceptors could explain its failure to attenuate the cardiovascular effects evoked by BHT-920 in anaesthetized normotensive rats (Fabeiro et al., 2000), although the contribution of the α_{2D} subtype to α_2-adrenoceptor-mediated pressor response in anaesthetized rats remains to be established.

Systematic studies undertaken to characterize prejunctional α_2-adrenoceptors in different species have shown that the predominant adrenoceptor on noradrenergic nerve terminals is the α_{2A}, whereas in rat it resembles the α_{2D}-adrenoceptor, a species orthologue of the human α_{2A}-adrenoceptor (Bylund et al., 1994; Docherty, 1998). As a rare exception to this postulate, prejunctional α_2-adrenoceptors in rat atrium were initially classified as B subtype (Connaughton and Docherty, 1990; Smith et al., 1992; Alberts, 1993); however, a reinvestigation of this unex-

pected finding showed that they might also be assigned to subtype D (Trendelenburg et al., 1997), or even to a mixture of both (Docherty, 1998). From the results of the present study, we feel that the antagonism exerted by (+)-boldine on sympathetic nerve endings in rat atrium ($pA_2 = 6.06$) is more attributable to α_{2B}- than to α_{2D}-adrenoceptor blockade, because (a) the affinity values found for (+)-boldine, BRL 44408 and ARC 239 ($pA_2 = 6.06$, $pEC_{30} = 6.49$ and 7.40, respectively; Smith et al., 1992; Table 2) in rat atrium were more than 30-fold different from those at guinea pig ileum α_{2D}-adrenoceptors ($pA_2 = 4.38$, 8.05 and 5.64, respectively); and (b) the values for BRL 44408 and ARC 239 previously determined in rat atrium ($pEC_{30} = 6.49$ and 7.40, respectively; Smith et al., 1992) better agree with the affinity values derived from the binding studies in tissues with relatively pure receptor populations of subtype B ($pK_i = 6.76$ and 8.34), than at subtype D ($pK_i = 7.80$ and 6.55) or subtype A ($pK_i = 8.44$ and 6.59, respectively; see Bylund et al., 1994), for the latter two BRL 44408 has been shown to be a more potent antagonist.

In radioligand binding studies with cloned human α_2-adrenoceptor subtypes A, B and C stably expressed in HEK cells, (+)-boldine behaved as a rather unselective antagonist of micromolar affinity, the affinity values obtained at α_{2A}- ($pK_i = 6.26$) and α_{2B}-adrenoceptors ($pK_i = 5.79$) were very close to the functional affinity values at rat and rabbit vas deferens α_2-adrenoceptors ($pA_2 = 6.02$ and 6.36, respectively) and at α_2-adrenoceptors in rat atrium ($pA_2 = 6.06$). In contrast, a consistently low affinity was found for (+)-boldine in binding studies at rat cortical α_{2D}-adrenoceptors ($pK_i = 4.70$), and functionally in guinea pig ileum ($pA_2 = 4.38$), confirming that the D subtype is the α_2-adrenoceptor involved in mediating the inhibition of acetylcholine in guinea pig ileum as previously suggested (Funk et al., 1995; Colucci et al., 1998). It would be interesting to further evaluate whether the antagonism at α_2-adrenoceptors by (+)-boldine does contribute to any of the broad ranges of its indications or, possibly, influences as yet undetected actions, like motor performance, mood and cognitive functions, in which the blockade of α_2-adrenoceptors has been shown to be favourable (Brefel-Courbon et al., 1998).

4.4. Vascular and cardiac effects

(+)-Boldine has been shown, although with approximately 200-fold lower binding affinity than at α_1-adrenoceptors, to interact with the benzothiazepine binding site of the Ca^{2+} entry complex in rat cerebral cortex labelled with [^{3}H]D-*cis*-diltiazem ($IC_{50} = 3.3 \times 10^{-5}$ M), but had no effect at the dihydropyridine binding site labelled with [^{3}H]nitrendipine ($IC_{50} > 10^{-4}$ M) (Ivorra et al., 1993a). In the present study, the Ca^{2+} channel antagonist property of (+)-boldine could be confirmed, because it inhibited the vasoconstriction in rat perfused kidney caused by elevated extracellular K^+ (27 mM) and was 70-fold weaker than diltiazem, a potency ratio being somewhat greater than that found earlier in rat uterus, where (+)-boldine proved to be 32-fold less potent than diltiazem to relax the tissue depolarized by high K^+ (56 mM) (Ivorra et al., 1993b). As deduced from its potency to inhibit a similarly strong vasoconstriction of 70–80% evoked by equieffective concentrations of noradrenaline (6×10^{-7} M) and KCl (27 mM) in perfused rat kidney ($-\log ED_{50}$ mol = 9.53 and 6.03, respectively), blockade by (+)-boldine of vascular α_{1A}-adrenoceptors exceeds that of Ca^{2+} channels by a factor of 3000, a potency difference that is considerably higher than that previously found for the drug (factor of 0.5 to 120) in rat aorta, rat small mesenteric artery and guinea pig aorta against noradrenaline or KCl-evoked vasoconstriction (Ivorra et al., 1993a; Chulia et al., 1996; Orallo et al., 1998; Fabeiro et al., 1999).

Since a common feature of most Ca^{2+} entry blockers, including diltiazem of the benzothiazepine type, is their capacity to inhibit the slow Ca^{2+} current through cardiac L-type Ca^{2+} channels (Janis et al., 1987), we used the guinea pig Langendorff heart, which allows to simultaneously study the influence of Ca^{2+} channel antagonists on the coronary vasculature and on the myocardium (Boddeke et al., 1987). In the present study, (+)-boldine, although being 50- to 60-fold weaker than diltiazem, showed a quite similar profile in terms of increasing coronary flow at concentrations not different from those acting negative inotropic. However, while heart rate was affected by diltiazem at approximately threefold higher concentrations ($IC_{25} = 2.5 \times 10^{-6}$ M) than those which already evoked negative inotropy ($IC_{50} = 9 \times 10^{-7}$ M), the reverse was observed for (+)-boldine, by which the negative chronotropic effect ($IC_{25} = 3 \times 10^{-5}$ M) exceeded its negative inotropy ($IC_{50} = 5.7 \times 10^{-5}$ M) approximately by a factor of two. The decrease in heart rate between 15% and 25% in perfused Langendorff hearts in response to lower concentrations of (+)-boldine (3×10^{-7}–3×10^{-5} M) was also observed in spontaneously beating right atria from guinea pig, with the response taking around 20 min to reach maximum after each individual concentration and more than 1 h to recover after removing the drug (not shown). Whereas in guinea pig Langendorff heart, the negative inotropic effect of different Ca^{2+} channel antagonists including that of diltiazem has been shown to solely rely on Ca^{2+} entry blockade without other actions, this may not be so for the negative chronotropic and coronary dilator actions of these compounds, for which additional mechanisms, like calmodulin antagonism, inhibition of adenosine uptake or an effect on sodium channels, have also been assigned to be involved (see Boddeke et al., 1987). Also in the case of (+)-boldine it appears that additional and as yet unknown factors besides "pure" Ca^{2+} entry blockade may contribute to the more pronounced negative chronotropic effect observed in Langendorff heart (and right atrium) from guinea pig. Interestingly, a significant bradycardia accompanying the long-lasting fall in mean arterial blood pressure has also

been found after intravenous administration of (+)-boldine (5 mg/kg) to anaesthetized rats (Orallo et al., 1998).

In guinea pig papillary muscle, (+)-boldine at a threshold concentration of 10^{-6} M mainly prolonged the action potential duration at higher levels of repolarization, thereby resembling class III antiarrhythmic agents like d-sotalol, and slightly increased the developed force of contraction. Maximal upstroke velocity was not affected by (+)-boldine, indicating a lack of inhibitory action on the ventricular sodium current. Relatively high concentrations (10^{-4} M) were needed to produce a negative inotropy in papillary muscle, an effect also being detectable in stimulated left and spontaneously beating right atria from guinea pig, in which exposure to (+)-boldine at concentrations between 10^{-6} and 3×10^{-5} M produced a weak positive inotropic response of maximally 15%, but a negative inotropy at threshold concentrations of 10^{-4} M (not shown). Interestingly, a reduction in APD_{30}, which is characteristic of the blockade of the Ca^{2+} current during the plateau phase of the action potential, was not observed with (+)-boldine in guinea pig papillary muscle, at least up to 10^{-5} M. Similar action potential prolonging but concomitantly force decreasing effects have been previously observed with diltiazem and verapamil in this preparation, their change has been discussed to be the result of differentially affecting the balance between suppressing effects on the slow inward Ca^{2+} current and on the outward K^+ current (Nakaya et al., 1988). Thus, in terms of the changes in the action potential configuration and the developed tension in guinea pig papillary muscle, (+)-boldine only partly resembles diltiazem. The ability of (+)-boldine to prolong the action potential could explain the slight positive inotropy by enhanced entry of Ca^{2+} through voltage-operated Ca^{2+} channels. Possibly, a Ca^{2+} channel blocking activity that (+)-boldine may possess at a concentration of 10^{-4} M could explain the shortening of action potential plateau at APD_{30} and the concomitant negative inotropic action. On the other hand, whether the action potential prolonging effect of (+)-boldine could be one reason for its more pronounced negative chronotropic effect, at concentrations not yet causing negative inotropy, observed in perfused guinea pig Langendorff hearts (and spontaneously beating right atrium) will remain unclear until further studies, e.g. electrophysiological experiments on the possible reduction by (+)-boldine of outward repolarizing currents in sino-atrial node and Purkinje fibre will have been performed to clarify this underlying mechanism.

4.5. Conclusion

In conclusion, the data from our functional and binding experiments at different α_1-adrenoceptors indicate that (+)-boldine has an approximately 25- and 15-fold higher affinity for the subtype A than for B and D, respectively. Additionally, we found (+)-boldine to be an almost equipotent antagonist of micromolar affinity at human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors, which is consistent with values obtained at non-human α_{2A}- and α_{2B}-adrenoceptors. Higher than micromolar concentrations were necessary to antagonize responses to stimulation of α_{1A}-adrenoceptors in its L-like state in rabbit spleen and of α_{2D}-adrenoceptors in guinea pig ileum and rat cerebral cortex. In vascular and cardiac preparations, (+)-boldine, although being 50- to 70-fold weaker than diltiazem, showed Ca^{2+} channel antagonistic properties but no specificity for coronary dilatation to cardiodepression.

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