Physiol. Res. 40: 481–491, 1991

The Beta-Adrenergic Receptor-Regulated 1,4-Dihydropyridine Calcium Channel Receptor Sites in Coronary Artery Smooth Muscle

J. DŘÍMAL

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava

Received December 10, 1990
Accepted June 26, 1991

Summary

The cross-regulatory communication from β-adrenergic receptors to 1,4-dihydropyridine (DHP) Ca^{2+} channel agonist and antagonist binding sites and cooperativity between DHP binding sites were studied in microsomal membranes of canine coronary artery (purified to a factor 2.9 for DHPs). The maximal number of binding sites (B_{max}) identified in coronary artery microsomal membranes (CAM) with Ca^{2+} channel agonist (−)-S-(^{3}H)BAY K 8644 was two times higher than B_{max} of sites labelled with Ca^{2+} channel antagonist (+)-(^{3}H)PN 200-110. The exposure of CAM to isoprenaline was accompanied with down-regulation of β-adrenergic receptors and with increase in binding capacity for DHPs. The increase in B_{max} was proportional in both groups of experiments and was related to increased affinity of DHPs. The 1,4-DHP binding sites identified in vascular smooth muscle showed characteristics typical for classification of specific 1,4-DHP receptor on Ca^{2+} channels. The binding was of high affinity, saturable and reversible, it showed stereoselectivity and it was positively modulated by β-adrenergic stimulation and it showed cAMP and GTP sensitivity. The results support the hypothesis that β-receptors also regulate the mode of Ca^{2+} channels in coronary artery smooth muscle.

Key words

Coronary artery – Smooth muscle – Microsomal membranes – L-type Ca^{2+} channels – (−)-S-(^{3}H)BAY K 8644 – (+)-(^{3}H)PN 200-110 – (−)-3-(^{125}I)iodocyanopindolol – Ligand binding

Introduction

Two types of calcium channels, similar to the Ca^{2+} channels in heart muscle have been found in cells from a variety of mammalian arteries (Bean *et al.* 1986, Benham *et al.* 1987) and in aortic cell lines (Cohen and McCarthy 1987, Friedman *et al.* 1986).

Considerable experimental evidence has accumulated in the last few years indicating that voltage-sensitive and possibly also receptor-regulated Ca^{2+} channels are regulated by cAMP and protein phosphorylation (Armstrong and Eckert 1987, Trautwein and Kameyama 1986). The Ca^{2+} channels in cardiac and in vascular smooth muscle, however, may not be precisely identical since the Ca^{2+} channels in

vascular smooth muscle are much more sensitive to 1,4-dihydropyridine Ca^{2+} channel antagonists (Bean *et al.* 1986) and possibly do not gain possession of that type of Ca^{2+} channel that is phosphorylated by cAMP-dependent protein kinase (Worley *et al.* 1986). Evidence has also emerged for direct coupling of G protein to Ca^{2+} channel (Trautwein and Herscheller 1990).

Recent studies have demonstrated the presence of specific, saturable and high-affinity binding sites for 1,4-dihydropyridines also in porcine (DePover *et al.* 1982, Muramatsu *et al.* 1985, Nishimura *et al.* 1987, Yamada *et al.* 1988, 1990) and in canine coronary arteries (Dřímal *et al.* 1988).

The purpose of the present study was to evaluate the cross-regulatory communication from β-adrenergic to 1,4-dihydropyridine Ca^{2+} channel binding sites in microsomal membranes of coronary artery smooth muscle. These studies were performed in an effort to examine further the effects of β-adrenergic receptor activation on cooperativity of 1,4-dihydropyridine Ca^{2+} channel agonist and Ca^{2+} channel antagonist receptor sites in microsomal membranes of coronary artery smooth muscle.

Methods

Male mongrel dogs (20–25 kg) were anaesthetized with sodium pentobarbital (30 mg/kg intravenously) and the hearts were rapidly removed. Segments of the large coronary arteries (*ramus anterior descendens* and *ramus circumflexus* of the left coronary artery) were dissected, cleaned from myocardial adipose and connective tissue, opened longitudinally and the intima and adventitia were carefully removed. The cleaned medial layers of the coronary artery were minced with scissors and homogenized in nine volumes of 0.35 mol/l of ice cold sucrose and 5 mmol/l of Tris-HCl (pH = 7.51).

Membrane preparation

Microsomal membranes of vascular smooth muscles were prepared according to Kwan *et al.* (1983), with the exception that the medial layers of large coronary arteries were prepared from postnuclear supernatant in accordance with Dřímal *et al.* (1987). Preparations were treated in an Ultraturrax homogenizer. Homogenates of the media were pressed under vacuum through stainless wire mesh and centrifuged further at 1500 x g for 10 min at 4 °C. The supernatant was centrifuged further at 105 000 x g for 45 min on a MOM Ultracentrifuge. The resulting pellet was resuspended in an assay buffer (50 mmol/l Tris-HCl, pH = 7.51) in a Potter-Elvehjem homogenizer and used as crude microsomal fraction. Refined microsomal fractions were prepared from the supernatant which sedimented in a sucrose buffer (8–60 % w/v sucrose gradient) at 130 000 x g using fixed angle rotor and centrifugation time of 60 min.

Enzyme assays

The measurements of 5'-nucleotidase and Mg^{2+}-ATPase activity were accomplished according to Kwan *et al.* (1984) and Pullman (1967). Spectrophotometric determinations were performed on a Beckman Model 25 double-beam spectrophotometer. The assay mixture for the Mg^{2+}-ATPase contained (in mmol/l): sucrose (0.25), Tris-acetate (50.0), EDTA (0.5), glucose (25.0), $MgCl_2$ (5.0), potassium-phosphate (20.0) and 1.5 x 10^4 cpm of ^{32}P (specific activity 5 mCi/g) and hexokinase Type 10 – 3 μg/mg of protein (70 μg). Protein was assayed by the method of Bradford (1976).

Binding assays

Equilibrium binding assays were carried at 25 °C (or in other experiments with change in temperature at 5 °C) in 450 μl volumes containing 50 mmol/l Tris-HCl (pH = 7.51) and 100 μl of membrane protein for 60 min (120 min). (−)-S-(^{3}H)BAY K 8644 and (+)-(^{3}H)PN 200-110 were added at concentration indicated in Fig. 1. The concentrations of various drugs or ions also present are indicated where appropriate. After incubation, the bound drug was separated from the free drug by rapid filtration through 25 mm Whatman GF/B filters followed by three consecutive 2.0 ml washes with the buffer at 20 °C (5 °C). The filters were placed in scintillation vials with 10 ml liquid scintillant (SLD, Spolana Neratovice).

To determine the dissociation constants by competition, binding was accomplished with 20 μg of membrane protein and with 0.75 nmol/l of (−)-S-(^{3}H)BAY K 8644 or with 1.12 nmol/l of (+)-(^{3}H)PN 200-110 for 50 min at 25 °C. Nonspecific binding values were obtained by addition of 1.0 μmol/l of unlabelled ligands or in further experiments by addition of 1.0 μmol/l of nimodipine. Ethanol at 0.5 % v/w did not change the binding.

For measurement of neural contamination of the refined microsomal fraction of the coronary artery smooth muscle (^{125}I)-ω-Conotoxin GVIA (concentration range from 0.1 to 100 pmol/l) was used. Nonspecific binding of unlabelled ligand in the medium were measured at a final concentration of 16.0 nmol/l. Saturation experiments were systematically performed as described previously (Dřímal *et al.* 1987, Dřímal 1989). The apparent dissociation constant (K_d) and B_{max} were estimated by the analysis of the saturation data according to Rosenthal (1967). Multiple receptor systems were analyzed using the affinity spectra method (Tobler and Engel 1983). All manipulations and procedures with 1,4-dihydropyridines were performed strictly under sodium light.

β-adrenergic receptor binding

(^{3}H)dihydroalprenolol (DHA) and (−)-3-(^{125}I)iodocyanopindolol were used for measurements of total number of β-adrenergic receptors. Specific binding of β-adrenergic ligands was measured by incubating (25 °C) microsomal membranes with the refined microsomal fraction for 30 min at various concentrations of β-adrenergic ligands (from 0.1 to 30 nmol/l) of (^{3}H)DHA and (−)-3-(^{125}I)iodocyanopindolol (up to 1.0 nmol/l); c-AMP content was assayed by the (^{125}I)-method (IRAPRA, Prague) as described previously (Dřímal 1989, 1991).

Chemicals

(−)-S-(^{3}H)BAY K 8644, specific activity 71 Ci/mmol, NEN Research Product (Dupont, Boston); (+)-(^{3}H)PN 200-110, specific activity 82 Ci/mmol, Amersham (Buckinghamshire, England); (−)-3-(^{125}I)iodocyanopindolol, specific activity about 2000 Ci/mmol, Amersham; (3-(^{125}I)iodotyrosyl^{22})ω-Conotoxin, specific activity about 2000 Ci/mmol, Amersham; (−)-isoprenaline hydrochloride (Fluka); BAY K 8644 (Calbiochem); verapamil (Knoll).

Results

Membrane characteristics and enzyme activity

The refined microsomal fractions (RM-1, RM-2 and RM-3) obtained from the continuous sucrose density gradient were morphologically more homogeneous than crude microsomes. When examined by electron microscopy, these fractions contained morphologically sealed membrane vesicles of various sizes, membrane fragments derived from the endoplasmatic reticulum (RM-1 and RM-3) and mitochondrial and other fragments of intracellular membranes. The RM-2 was much more homogeneous.

Table 1

Enzyme activity of postnuclear and microsomal membrane fractions isolated from the media of canine coronary arteries by differential centrifugation and sucrose density gradient centrifugation

Fraction Sucrose density gradient	PNS	P	CM	RM-1 10/15	RM-2 20/30	RM-3 30/40	SOL
5'-nucleotidase	1.5 ±0.04	1.6 ±0.09	3.8 ±0.1	4.4 ±0.9	8.9 ±1.7	1.2 ±0.5	0.8 ±0.6
Mg^{2+}-ATPase (μmol/mg/h)	8.0 ±0.1	10.0 ±0.9	25.0 ±9.0	33.0 ±17.0	96.0 ±19.0	6.0 ±4.0	0.5 ±4.3

Values are Means ± S.E.M. (n=5); PNS – postnuclear supernatant, P – particulate, CM – crude microsomal fraction, RM-1 to RM-3 – refined microsomal fractions, SOL – soluble fractions

Table 2

Effect of isoprenaline of cAMP content in microsomal membranes of coronary artery

	cAMP content (pmol/mg protein)
Control	0.85±0.09
Isoprenaline	5.08±0.50

Values are Means ± S.E.M. (n=8)

Enzymatically RM-2 fraction (Tab. 1) showed the highest marker enzyme activity of two major membrane enzyme markers 5'-nucleotidase and Mg^{2+}-ATPase. The adenylate-cyclase activity is summarized in Tab. 2. Short-term incubation of the left coronary artery preparation (*ramus circumflexus*) with isoprenaline (1.0 μmol/l; n = 4) significantly increased the cAMP content in RM-2 fraction obtained from that preparation.

β-adrenoreceptor numbers in microsomal membranes following exposure to isoproterenol

To elucidate the effects of prolonged β-adrenoreceptor stimulation in the coronary artery on the number of microsomal membrane receptors we compared the ability of β-adrenergic ligands ((^3H)DHA and (−)-3-(^{125}I)iodocyanopindolol) to bind to microsomal membranes prepared from control unexposed coronary arteries and from those exposed to isoprenaline for one hour.

We have studied β-adrenergic receptor in the coronary artery using two β-adrenergic ligands. Saturation binding experiments with (^3H)DHA under control

conditions showed K_d 5.2±0.2 nmol/l and B_{max} 9.60±0.90 fmol/mg protein. The exposure of coronary artery preparations to isoprenaline significantly reduced (−)-3-(^{125}I)iodocyanopindolol specific binding from 13.4±0.88 fmol/mg protein to 8.9±1.2 fmol/mg protein (n = 10) while the (−)-3-(^{125}I)iodocyanopindolol affinity for this binding site was unaltered averaging 6.21±0.16 nmol/l.

Identification of 1,4-dihydropyridine Ca^{2+} channel antagonist binding sites in microsomal membranes

Specific binding of Ca^{2+} channel agonist to microsomal membranes (hereafter referred to as membrane inasmuch as only the RM-2 fraction was used in 1,4-dihydropyridine binding experiments) was rapid, reaching steady state within 20 min (at 25 °C). This steady state was maintained for at least 70 min. The calculated association rate measured in kinetic experiments using 0.90 nmol/l of (−)-S-(^{3}H)BAY K 8644 was 0.31 min^{-1}. The microsomal membranes in our experiments had a purification factor 2.1 for β-adrenergic radioligands and 2.9 for 1,4-dihydropyridines.

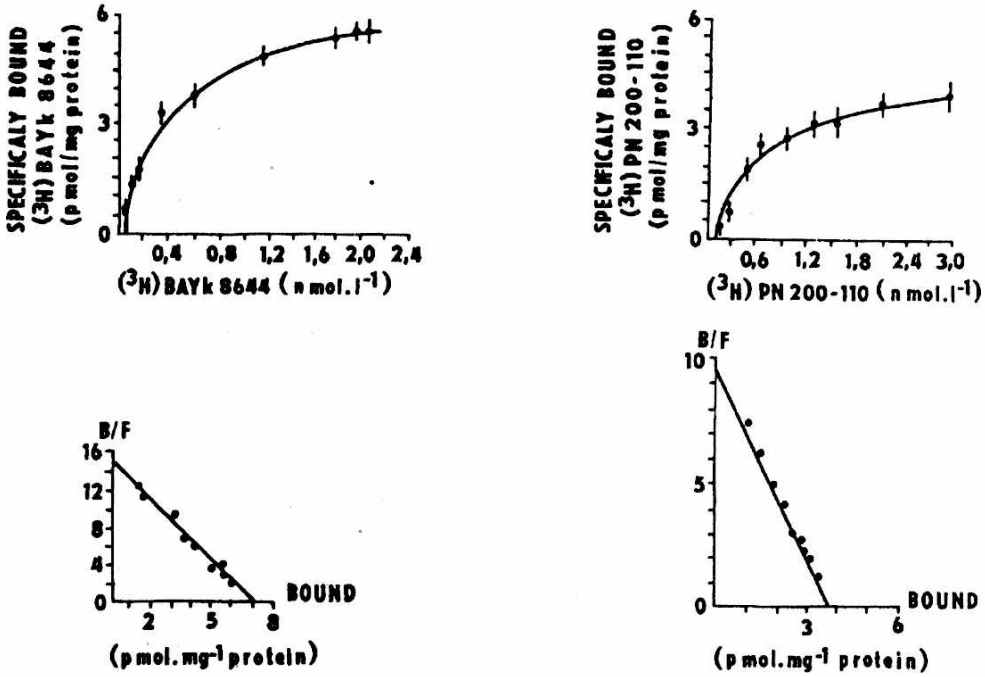

Fig. 1

Upper part: Specific (−)-S-(^{3}H)BAY K 8644 and (+)-(^{3}H)PN 200-110 binding in coronary artery smooth muscle microsomal membranes as a function of maximal concentration of the ligand. Nonspecific binding was detected in the presence of 1 μmol/l of unlabelled 1,4-dihydropyridine in triplicate assays. Ordinate - specific binding (pmol/mg protein), abscissa - free concentration of 1,4-dihydropyridine (nmol/l). Lower part: Scatchard plot derived from specific binding of Ca^{2+} channel agonist and Ca^{2+} channel antagonist. Ordinate - bound over free (B/F), abscissa - bound 1,4-dihydropyridine (pmol/mg protein). Note the prevalence of high affinity binding sites for 1,4-dihydropyridine Ca^{2+} channel in fragmented tissue.

The binding of (−)-S-(^{3}H)BAY K 8644 and (+)-(^{3}H)PN 200-110 to partially purified microsomal membranes was specific and saturable over a concentration range from 0.14 to 2.4 (or 0.14 to 3.0) nmol/l, respectively (Fig. 1). The linear regression analysis of untransformed experimental data showed adequate affinity and relatively high capacity of binding sites with the corresponding B_{max} values 7.15±0.04 and 3.40±0.18 pmol/mg protein and K_d values 18.0±0.1 and 9.0±0.8 nmol/l, respectively. The Scatchard analysis in each group of experiments was linear, indicating that a single population of high affinity binding sites was present.

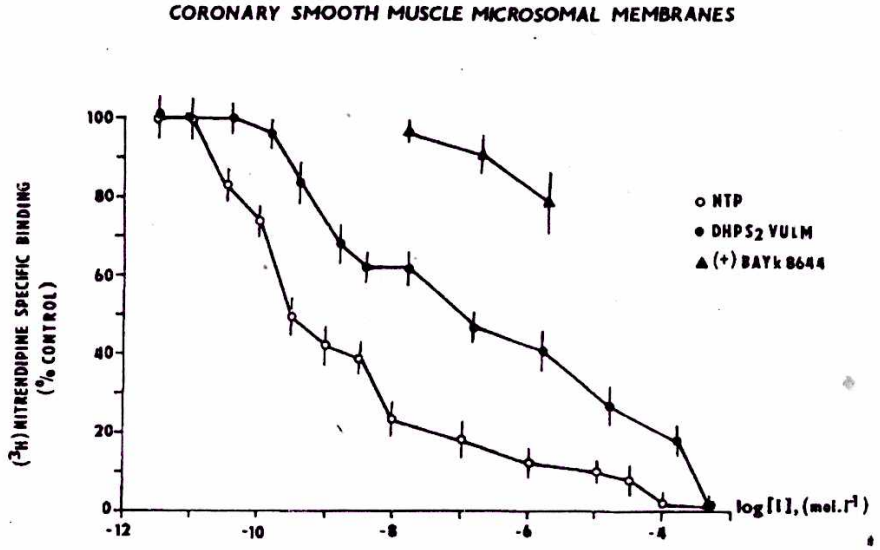

Fig. 2
Displacement experiments using (^{3}H)nitrendipine (1.1 nmol/l) and unlabelled displacers nitrendipine (NTP), nimodipine derivative DHPS-2 VULM and enantiomer of BAY K 8644. Total binding is plotted against $-\log_{10}$ of displacer (I). Data are the means ± S.E.M. of five experiments, each in triplicate, using coronary smooth muscle microsomal membranes.

The displacement studies in competition experiments showed effective displacement of nitrendipine by unlabelled nitredipine and by an analogue of nimodipine DHPS-2 VULM (Fig. 2). The K_i values calculated from concentrations that inhibited specific (−)-S-(^{3}H)BAY K 8644 binding by 50 % for optical isomers of BAY K 8644 (data not shown) displayed stereoselectivity for the optic isomer of (−)-S-(^{3}H)BAY K 8644.

Effects of drugs on (−)-S-(^{3}H)BAY K 8644 and (+)-(^{3}H)PN 200-110 specific binding in microsomal membranes

The effects of structurally unrelated Ca^{2+} channel antagonist and of pharmacologically active drugs on 1,4-dihydropyridine Ca^{2+} channel agonist and Ca^{2+} channel antagonist binding were studied to investigate further the cooperativity of interactions between 1,4-dihydropyridine binding sites and modulatory effects of different pharmacologically active drugs on 1,4-dihydropyridine binding. Additional evidence for the existence of the

modulatory role of basic cellular signals in the regulations of 1,4-dihydropyridine Ca^{2+} channel receptor was obtained in experiments with four different procedures: β-adrenergic receptor stimulation, GTP, cAMP and EDTA presence in the system.

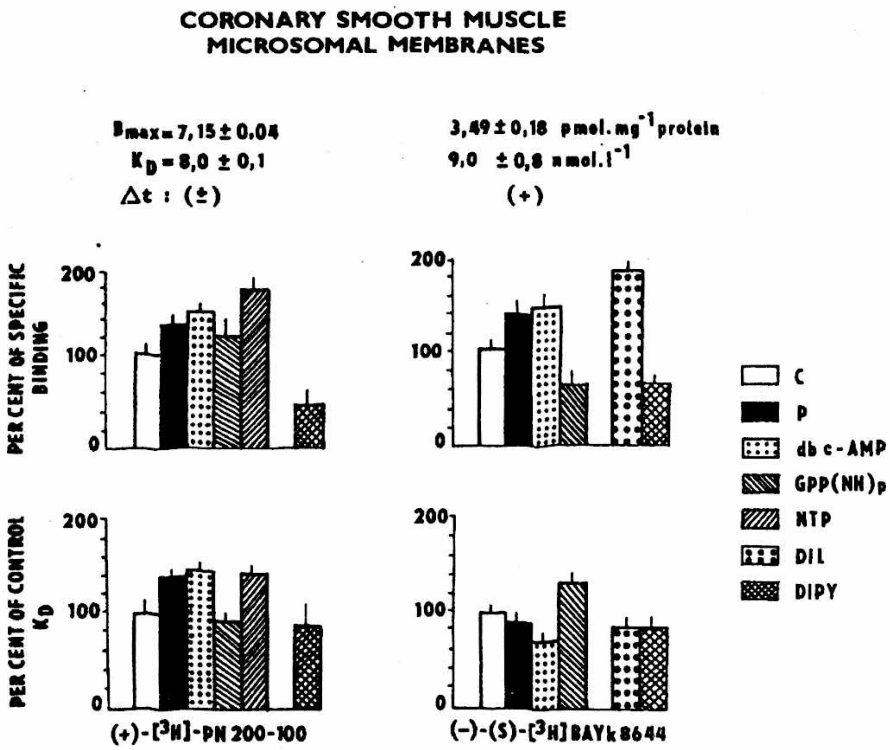

Fig. 3
Pharmacology of 1,4-dihydropyridine Ca^{2+} channel antagonist (+)-(^{3}H)PN 200-110 and Ca^{2+} channel agonist (−)-S-(^{3}H)BAY K 8644 binding in coronary artery smooth muscle microsomal membranes. The concentration of (+)-(^{3}H)PN 200-110 and (−)-S-(^{3}H)BAY K 8644 binding sites after incubation of coronary artery preparation with isoprenaline (P, 1.0); dibutyryl-cAMP (db cAMP, 1.0); guanylylimidodiphosphate (Gpp(NH)p, 10.0); nitrendipine (NTP, 1.0); d-cis-dilthiazem (DIL, 10.0) and dipyridamole (DIPY, 100.0); all in μmol/l.

As is shown in Fig 3, the exposure of a coronary artery preparation to isoprenaline was accompanied by increased density of (−)-S-(^{3}H)BAY K 8644 and (+)-(^{3}H)PN 200-110 binding. The increase in B_{max} was proportional in both groups of experiments (+56±7 and +40±9 %) and was mostly due to the increased affinity of both 1,4-dihydropyridines for its receptors (K_d = 10.9±1.1 and 7.0±0.2 nmol/l, i.e. a reduction by −39±8 and −22±6 %, respectively, $P<0.05$). The involvement of G protein in observed response was characterized after pretreatment of membranes with guanylylimidodiphosphate, a nonhydrolyzable analogue of GTP. This pretreatment significantly decreased the maximal capacity of (−)-S-(^{3}H)BAY K 8644 binding (−37±8 %, $P<0.05$). The nonhydrolyzable analogue of cAMP, dibutyryl-cAMP, significantly increased (−)-S-(^{3}H)BAY K 8644 maximal binding and increased affinity of Ca^{2+} channel agonist for its receptor (32±6 %). The non-1,4-DHP Ca^{2+} channel antagonist d-cis-dilthiazem significantly

increased 1,4-dihydropyridine Ca^{2+} channel receptor binding (+81±11 %). The presence of chelator EDTA in the system significantly reduced 1,4-dihydropyridine binding (−87±5 %, $P<0.05$). Adenosine uptake inhibitor, dipyridamole, significantly reduced maximal capacity of (−)-S-(^{3}H)BAY K 8644 binding (−59±5 %, $P<0.05$).

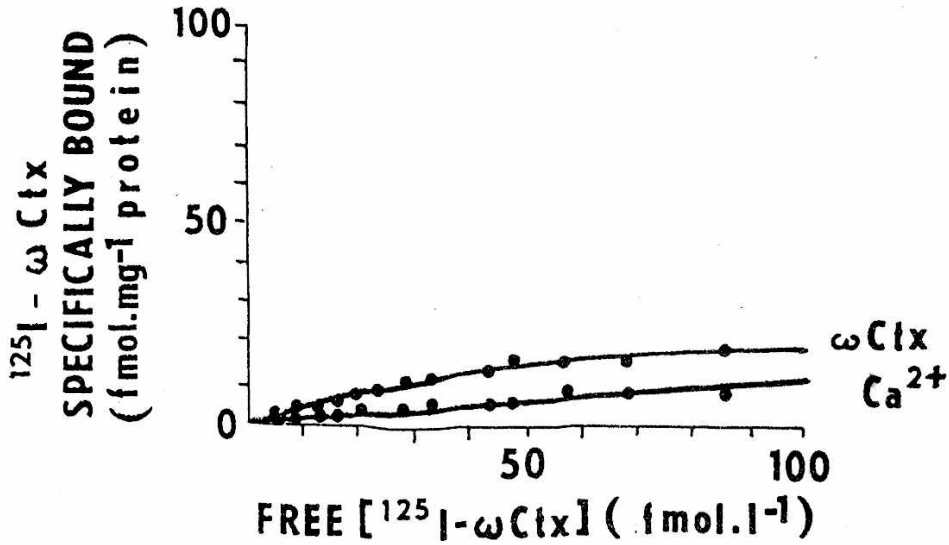

Fig. 4
Equilibrium binding of (^{125}I)-ω-Conotoxin to microsomal membranes of the coronary artery. Each value of this graph represents the mean of six experiments. Binding of (^{125}I)-ω-Conotoxin (pmol/l) in the controls and in the presence of 1 μmol/l $CaCl_2$ (lower curve).

The experiments with (^{125}I)-ω-Conotoxin GVIA revealed that microsomal membranes of coronary artery smooth muscle may contain small, but significant component of neural membrane fragments (Fig. 4).

Discussion

In the present study we have used optically pure enantiomers of the chiral molecule of 1,4-dihydropyridine Ca^{2+} channel ligands to demonstrate the presence and the modulation of 1,4-dihydropyridine Ca^{2+} channel agonist and Ca^{2+} channel antagonist binding sites in smooth muscle membrane of the coronary artery. The β-adrenergic receptor activation is shown to correlate with an increased levels of cAMP, with the down-regulation of membrane bound β-adrenergic receptors and with an increased total number of 1,4-dihydropyridine Ca^{2+} channel agonist and Ca^{2+} channel antagonist binding sites in microsomal membranes of the coronary artery.

The total number of binding sites labelled with the Ca^{2+} channel agonist in microsomal membranes was more than two times higher that the number of binding sites identified with the Ca^{2+} channel antagonist. Binding sites identified with 1,4-dihydropyridine Ca^{2+} channel agonist (−)-S-(^{3}H)BAY K 8644 showed a high affinity, saturability and reversibility, were stereoselective and showed a positive modulation with β-adrenergic activation of the system, and displayed GTP and cAMP sensitivity. The Ca^{2+} channel antagonist (+)-(^{3}H)PN 200-110 binding showed a high affinity, saturability in microsomal membranes and positive modulation with β-adrenergic receptor stimulation but it did not display GTP and cAMP sensitivity.

Compared to our data on 1,4-dihydropyridine binding in the coronary artery membrane preparation, lower B_{max} values were reported for (^{3}H)nitrendipine (561 fmol/mg protein) by Nishimura *et al.* (1987) and for (+)-(^{3}H)PN 200-110 in porcine coronary artery membrane (Yamada *et al.* 1988, 1990, Muramatsu *et al.* 1985). These discrepancies could be due to species differences and/or the employment of different preparative procedures.

The goal of the present report was to study the systematic difference in the regulatory response of Ca^{2+} channel agonist and antagonist binding in the presence of high levels of GTP and cAMP in microsomal membranes. Contrary to the β-adrenergic receptor stimulation resulting in a positive modulation of 1,4-dihydropyridine binding in both our groups, the presence of nonhydrolyzable analogue of cAMP and GTP in other experiments failed to induce any significant change in the B_{max} of antagonist. This finding may further support the hypothesis of a modal mode of Ca^{2+} channel.

The β-adrenergic receptor stimulation will increase both 1,4-dihydropyridine Ca^{2+} channel agonist and Ca^{2+} channel antagonist binding sites in microsomal membranes but in the presence of high levels of cAMP in microsomal membranes only 1,4-dihydropyridine Ca^{2+} channel agonist binding is increased by the "activation" of Ca^{2+} channels.

As in the cardiac muscle, the slowly inactivating type of Ca^{2+} channel in coronary vascular smooth muscle is sensitive to the activator BAY K 8644. Hughes *et al.* (1990) recently reported that agonist and antagonist enantiomers of 1,4-dihydropyridine PN 202-791 may act at different sites on the voltage-dependent calcium channel of vascular smooth muscle.

A small but significant component of specific ω-Conotoxin binding in microsomal membranes suggests the complexity of different "subtypes" of Ca^{2+} channels expressed in the coronary artery.

The allosteric regulation of 1,4-dihydropyridine binding with d-cis-dilthiazem, namely the stimulation of 1,4-dihydropyridine binding, has been demonstrated in various membrane preparations in different laboratories (DePover *et al.* 1982, Yamamura *et al.* 1982, Ferry *et al.* 1982, Vaghy *et al.* 1987). In the present study we document very prominent stimulation of 1,4-dihydropyridine Ca^{2+} channel agonist binding in smooth muscle membranes of coronary arteries.

With findings of a profound inhibition of 1,4-dihydropyridine binding in microsomal membranes after pretreatment with an adenosine uptake blocker, dipyridamole, we confirm our recent results (Dřímal 1988) suggesting that the pharmacological receptor for 1,4-dihydropyridines in coronary artery smooth muscle membranes is associated with a nucleoside carrier.

The findings that 1,4-dihydropyridine Ca^{2+} channel antagonist stabilizes Ca^{2+} channel binding sites in the coronary artery smooth muscle membrane in an inactive mode and that β-adrenergic receptor activation exerts modulatory effects on 1,4-dihydropyridine Ca^{2+} channel binding, may have important implications for understanding the mechanism of action of 1,4-dihydropyridine drugs on coronary artery smooth muscle.

Acknowledgement

The author would like to thank Mrs. B. Sedlarova and Mrs. Z. Veghova for their technical assistance.

References

ARMSTRONG D., ECKERT R.: Voltage-activated calcium channels that must be phosphorylated to respond to membrane depolarization. *Proc. Natl. Acad. Sci. USA* **84:** 2518–2522, 1987.

BEAN B.P., STUREK M., PUGA A., HERMSMEYER K.: Calcium channels in muscle cells isolated from rat mesenteric arteries. Modulation by dihydropyridine drugs. *Circ. Res.* **59:** 229–235, 1986.

BENHAM C.A., TSIEN R.W.: A novel-receptor-operated Ca^{2+} permeable channel activated by ATP in smooth muscle. *Nature* **328:** 275–278, 1987.

BRADFORD M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72:** 248–254, 1976.

COHEN C.J., MCCARTHY R.T.: Nimodipine block of calcium channels in rat vascular smooth muscle cells. *J. Gen. Physiol.* **387:** 2412–2416, 1987.

DEPOVER A., MATLIB M.A., LEE S.W., DUBE G.P., GRUPP G., SCHWARTZ A.: Specific binding of (^{3}H)nitrendipine to membranes from coronary arteries and heart in relation to pharmacological effects. Paradoxical stimulation by dilthiazem. *Biochem. Biophys. Res. Commun.* **108:** 110–117, 1982.

DŘÍMAL J.: Regulation of β-adrenergic receptors and calcium channel agonist binding sites in cultured human embryonal smooth muscle cells. *Gen. Physiol. Biophys.* **8:** 341–350, 1989.

DŘÍMAL J.: Heterogeneity of calcium channel agonist binding sites in the coronary artery. *Gen. Physiol. Biophys.* **7:** 135–142, 1988.

DŘÍMAL J.: Regulation of 1,4-dihydropyridine and β-adrenergic receptor sites in coronary artery smooth muscle membranes. *Cell Signalling* **3:** 225–232, 1991.

DŘÍMAL J., KNEZL V., MAGNA D., STŘÍŽOVÁ K.: External transport of β-adrenergic binding sites in ischemic myocardium. *Gen. Physiol. Biophys.* **6:** 583–591, 1987.

DŘÍMAL J., MAGNA D.,KNEZL V., SOTNIKOVÁ R.: Evidence that high affinity (^{3}H)clonidine binding cooperates with H_2-receptors in the canine coronary smooth muscle membrane. *Agents Action* **23:** 263–265, 1988.

FERRY D.R., GLOSSMANN H.: Evidence for multiple receptor sites within the putative calcium channel. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **321:** 80–83, 1982.

FRIEDMAN M.E., SUAREZ-KURTZ S., KAIZOWSKI G., KATZ G.M., REUBEN J.P.: Two calcium currents in a smooth muscle cell line. *Am. J. Physiol.* **250:** H699–H703, 1986.

HUGHES A.D., HERING S., BOLTON T.B.: Evidence that agonist and antagonist enantiomers of the dihydropyridine PN 202-709 act at different sites on the voltage-dependent calcium channel of vascular smooth muscle. *Br. J. Pharmacol.* **101:** 3–5, 1990.

KWAN C.Y., TRIGGLE C.R., GROOVER A.K., LEE R.M.K.W.: An analytical approach to the preparation and characterization of subcellular membranes from canine mesenteric arteries. *Preparat. Biochem.* **13:** 275–314, 1983.

KWAN C.Y., TRIGGLE C.R., GROOVER A.K., LEE R.M.K.W., DANIEL E.E.: Membrane fractionation of canine aortic smooth muscle: subcellular distribution of calcium transport activity. *J. Mol. Cell. Cardiol.* **16:** 747–764, 1984.

MURAMATSU M., FUJITA A., TANAKA M., AIHARA H.: Effects of neuraminidase on dilthiazem-mediated alteration of nitrendipine binding in the coronary artery. *Jpn. J. Pharmacol.* **39**: 217–223, 1985.

NISHIMURA J., KANAIDE H., NAKAMURA M.: Characteristics of adrenoceptors and (^{3}H)nitrendipine receptors of porcine vascular smooth muscle. Differences between coronary artery and aorta. *Circ. Res.* **60**: 837–844, 1987.

PULLMANN M.E.: Measurement of ATPase, ^{14}C-ADP-ATP, and $^{32}P_i$-ATP exchange reactions. *Meth. Enzymol.* **10**: 57–60, 1967.

ROSENTHAL H.E.: Graphic method for the determination and presentation of binding parameters in a complex system. *Anal. Biochem.* **20**: 525–531, 1967.

TOBLER H.J., ENGEL J.: Affinity spectra: a novel way for the evaluation of equilibrium binding experiments. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **322**: 187–192, 1983.

TRAUTWEIN W., HERSCHELLER J.: Regulation of cardiac L-type of calcium current by phosphorylation and G proteins. *Annu. Rev. Physiol.* **52**: 257–274, 1990.

TRAUTWEIN W., KAMEYAMA M.: Beta-adrenergic control of calcium channels in cardiac myocytes. In: *Calcium Electrogenesis and Neuronal Functioning.* U. HEINEMANN, M. KLEE, E. NEHER, W. SINGER (eds), Exp. Brain Res. Ser. Vol. 14, Springer, Berlin, 1986, pp. 185–195.

VAGHY P.L., WILLIAMS J.S., SCHWARTZ A.: Receptor pharmacology of calcium entry blocking agents. *Am. J. Cardiol.* **59**: 4A–17A, 1987.

WORLEY J.F., DEITMAR J.W., NELSON M.T.: Single nisoldipine-sensitive calcium channels in smooth muscle cells isolated from rabbit mesenteric artery. *Proc. Natl. Acad. Sci. USA* **83**: 5746–5750, 1986.

YAMADA S., HARADA Y., NAKAYAMA K.: Characterization of Ca^{2+} channel antagonist binding sites labelled by (^{3}H)nitrendipine in coronary artery and aorta. *Eur. J. Pharmacol.* **154**: 203–208, 1988.

YAMADA S., KIMURA K., HARADA Y., NAKAYAMA K.: Ca^{2+} channel receptor sites for (+)-(^{3}H)PN 200-110 in coronary artery. *J. Pharmacol. Exp. Ther.* **252**: 327–332, 1990.

YAMAMURA H.I., SCHOEMAKER H., BOLES R.G., ROESKE W.R.: Diltiazem enhancement of [^{3}H]nitrendipine binding to calcium channel associated drug receptor sites in rat brain synaptosomes. *Biochem. Biophys. Res. Commun.* **108**: 640–646, 1982.

Reprint requests

Dr. J. Dřímal, Institute of Experimental Pharmacology, Slovak Academy of Sciences, CS–842 16 Bratislava, Dúbravská cesta 2.