(−)-[^{3}H]Desmethoxyverapamil, a novel Ca^{2+} channel probe

Binding characteristics and target size analysis of its receptor in skeletal muscle

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(−)-[^{3}H]Desmethoxyverapamil (2,7-dimethyl-3-(3,4-dimethoxyphenyl)-3-cyan-7-aza-9-(3-methoxyphenyl)-nonanhydrochloride) was used to label putative Ca^{2+} channels in guinea pig skeletal muscle. The binding sites for (−)-[^{3}H]desmethoxyverapamil co-purified with t-tubule membrane markers in an established subcellular fractionation procedure. (−)-[^{3}H]Desmethoxyverapamil bound to partially purified t-tubule membranes with a K_D of 2.2 ± 0.1 nM and a B_{max} of 18 ± 4 pmol/mg membrane protein at 25°C. Binding was stereoselectively inhibited by phenylalkylamine Ca^{2+} antagonists and in a mixed, non-competitive fashion by the benzothiazepine Ca^{2+} antagonist d-*cis*-diltiazem and the 1,4-dihydropyridine Ca^{2+} antagonist (+)-PN 200-110. Target size analysis of the (−)-[^{3}H]desmethoxyverapamil drug receptor site revealed a molecular mass of 107 ± 2 kDa. In contrast, the target size of the allosterically coupled benzothiazepine drug receptor site, labelled by d-*cis*-[^{3}H]diltiazem, was 130.5 ± 4 kDa ($p < 0.01$) and of the 1,4-dihydropyridine binding site 179 kDa, when labelled with [^{3}H]nimodipine. It is concluded that (−)-[^{3}H]desmethoxyverapamil is an extremely useful radioligand for the phenylalkylamine-selective receptor site of the t-tubule localized Ca^{2+} channel which is allosterically linked to two other distinct drug receptor sites.

(−)-[^{3}H]Desmethoxyverapamil *Skeletal muscle* *Ca^{2+}-channel* *Target size*

1. INTRODUCTION

The inward Ca^{2+} current of chordate skeletal muscle t-tubules is blocked by Ca^{2+} antagonists of the 1,4-dihydropyridine (nifedipine), phenylalkylamine (D-600) [1] and benzothiazepine (d-*cis*-diltiazem) [2] classes. Putative skeletal muscle Ca^{2+} channels were first labelled directly by the 1,4-dihydropyridine Ca^{2+} antagonist [^{3}H]nimodipine [3] and the structurally unrelated d-*cis*[^{3}H]-diltiazem [4]. Subcellular fractionation experiments have shown that the high affinity binding sites for labelled 1,4-dihydropyridines in skeletal muscle reside almost exclusively in membranes derived from t-tubules [5,6]. Another drug receptor site, which is selective for phenylalkylamine Ca^{2+} antagonists, has been recently labelled in t-tubule membranes with (±)-[^{3}H]verapamil [7,8]. The K_D value of (±)-[^{3}H]verapamil is, however, high (30–40 nM) which severely limits the usefulness of this channel probe. An optically pure verapamil derivative, (−)-desmethoxyverapamil, has been shown to be 20-times more potent than verapamil in functional studies [9] and with respect to receptor affinity in brain [10]. Here we describe the subcellular distribution of (−)-[^{3}H]desmethoxyverapamil binding sites in skeletal muscle, their allosteric regulation through two other distinct drug receptor sites of the Ca^{2+} channel and the molecular mass determination of the (−)-[^{3}H]-desmethoxyverapamil drug receptor site with the aid of target size analysis.

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2. MATERIALS AND METHODS

2.1. *Materials*

(−)-[^{3}H]Desmethoxyverapamil (83 and 7.2 Ci/mmol), the unlabelled enantiomers of desmethoxyverapamil (code names (+)- or (−)-D-888), D-600 and the biologically less potent phenylalkylamine derivative (±)-D-619 were gifts from Knoll AG; d-*cis*-[^{3}H]diltiazem (83 Ci/mmol), unlabelled d-*cis*-diltiazem and l-*cis*-diltiazem were gifts from Goedecke AG, (+)-[^{3}H]PN 200-110 (bindability ⩾ 85%; 75 Ci/mmol) was from Amersham. (−)-[^{3}H]Dihydroalprenolol (92 Ci/mmol) was from New England Nuclear. [^{3}H]Nimodipine (145 Ci/mmol) was from Bayer. PN 200-110 enantiomers were from Sandoz.

2.2. *Membrane purification*

Partially purified skeletal muscle t-tubule membranes were prepared as in [3]. Subfractionation of the partially purified t-tubule membranes was performed with a discontinuous sucrose density gradient as in [5].

2.3. *Binding assays*

These are conducted for (−)-[^{3}H]desmethoxyverapamil in a volume of 0.25 ml in 50 mM Tris–HCl, supplemented with 0.1 mM phenylmethylsulfonylfluoride (pH 7.4) at 25°C, allowing 1 h before bound and free radioactivity are separated through Whatman GF/C filters as described in [3]. Binding-inhibition experiments with unlabelled drugs were performed with 1–2 nM of the 83 Ci/mmol (−)-[^{3}H]desmethoxyverapamil and 10–20 μg of partially purified t-tubule membrane protein. (−)-D-600 and (−)-desmethoxyverapamil displaced to the same level of binding when present at an approx. 1000-fold molar excess over 2 nM (−)-[^{3}H]desmethoxyverapamil. In all experiments 10 μM (−)-D-600 or 1 μM (−)-desmethoxyverapamil defined 'blank binding', which was subtracted from total binding to give specific binding. For saturation experiments, high and low specific activity (−)-[^{3}H]desmethoxyverapamil were employed to allow examination of the saturation isotherm over a wide range of free ligand concentrations (0.05 to ~40 × K_D). To evaluate the nature of competition of various drugs, (−)-[^{3}H]des- methoxyverapamil saturation isotherms were constructed (with the 83 Ci/mmol tracer over the range 0.1 to ~4 × K_D) in the absence and presence of unlabelled drugs. [^{3}H]Nimodipine [3], d-*cis*-[^{3}H]diltiazem binding [4] and [^{3}H]ouabain binding [3,5,7] was performed as previously described. Binding–inhibition curves were fitted with non-linear curve fitting [11] and the kinetics of association with the differential form of the second-order rate equation [3].

2.4. *Target size analysis*

Partially purified skeletal muscle t-tubule membranes were irradiated at −110°C with 10 MeV electrons at a dose rate of 2 Mrad/min at a protein concentration of 2–3 mg/ml [7,12–14]. Radiation doses were measured with radiochromic dye films (Far West Technology, Goleta, USA). Following irradiation, membranes were stored in liquid nitrogen for 24 h, then thawed and diluted 1:30 for (−)-[^{3}H]desmethoxyverapamil binding at 0.2 or 2.0 nM, 1:20 for [^{3}H]nimodipine binding (at 2 nM) or 1:10 for d-*cis*-[^{3}H]diltiazem binding (at 4 nM). We have shown elsewhere that the affinities for [^{3}H]nimodipine [12,14] and (±)-[^{3}H]verapamil [7], for residual binding sites are not affected by irradiation. The same is found for (−)-[^{3}H]desmethoxyverapamil and d-*cis*-[^{3}H]diltiazem binding. The M_r values of the irradiated targets were calculated according to the relationship derived in [15] with a temperature correction factor (f) of 2.8 (see [16]),

$$M_r = f\left[\frac{6.4 \times 10^5}{D_{37}\ [\text{Mrad}]}\right]$$

where D_{37} is the dose of radiation, which reduces the binding of a given ligand to 37% of the value in a non-irradiated control.

3. RESULTS

3.1. *Subcellular distribution of (−)-[^{3}H]desmethoxyverapamil binding sites*

Previously it was shown that the binding sites for the Ca^{2+} antagonist [^{3}H]nimodipine in the microsomal fraction of guinea pig skeletal muscle can be further purified by discontinuous sucrose density gradient centrifugation [5]. Membranes, collecting at the interface between 25% (w/w) sucrose and the overlay, have a specific density of ~60000 fmol of 1,4-dihydropyridine receptor sites per mg protein. On the basis of protein and

phosphoprotein composition, electronmicroscopy and marker enzymes, this fraction appears to be mainly of t-tubule origin. In table 1 a representative experiment of the subfractionation procedure is shown. Clearly, the (−)-[^{3}H]desmethoxyverapamil sites copurify with the other Ca^{2+} channel markers and, as previously shown for [^{3}H]nimodipine [5,7], (±)-[^{3}H]verapamil and d-*cis*-[^{3}H]diltiazem [7], have the highest specific density in the t-tubule membrane where β-adrenoceptors are also enriched.

3.2. *Saturation isotherm and association kinetics of (−)-[^{3}H]desmethoxyverapamil*

The association reaction of (−)-[^{3}H]desmethoxyverapamil with its binding sites followed a rapid time course at 25°C. Employing 1–2 nM (−)-[^{3}H]desmethoxyverapamil and 0.3–0.5 nM drug receptor sites, a steady state is reached within 45 min (fig.1A). Specific binding was fully reversible (not shown). The saturation isotherm of (−)-[^{3}H]desmethoxyverapamil binding is monophasic with a K_D of 2.0 ± 0.1 nM and B_{max} of 18 ± 4.0 pmol/mg protein at 25°C (n = 10). An example of a typical experiment is shown in fig.1B. At the K_D concentration of free (−)-[^{3}H]desmethoxyverapamil ~95% filter-retained radioactivity is specific binding.

3.3. *Interaction of (−)-[^{3}H]desmethoxyverapamil labelled sites with Ca^{2+} channel antagonists*

(−)-[^{3}H]Desmethoxyverapamil binding is inhibited stereoselectively by the phenylalkylamine Ca^{2+} antagonists (−)- and (+)-desmethoxyverapamil and (−) and (+)-D-600 (see fig.2). The binding inhibition profile for the 1,4-dihydropyridines (+)-PN 200-110 and (−)-PN 200-110 was clearly stereoselective, however (+)-PN 200-110 was only partially inhibitory up to 100 nM (fig.2) even at very low ^{3}H-ligand concentrations. Benzothiazepines also interacted with the (−)-[^{3}H]desmethoxyverapamil labelled sites stereoselectively. The IC_{50} value of d-*cis*-diltiazem was 30-times lower than that of the biologically inactive [17] l-*cis*-diltiazem. The mode of competition was evaluated for the non-phenylalkylamine Ca^{2+} antagonists d-*cis*-diltiazem (fig.3) and the 1,4-dihydropyridine (+)-PN 200-110 (not shown); d-*cis*-diltiazem and (+)-PN 200-110 increased, to a limited extent, the apparent K_D and decreased the B_{max} for (−)-[^{3}H]desmethoxyverapamil, which is qualitatively the same result found for (−)-

Table 1

Purification of t-tubule membranes from the guinea pig hind limb skeletal muscle

	Calcium channel labels								Plasma membrane markers			
	(+)-[^{3}H]-PN 200-110		[^{3}H]-Nimodipine		(−)-[^{3}H]-Desmethoxy-verapamil		d-*cis*-[^{3}H]-Diltiazem		Na^+K^+-ATPase label [^{3}H]ouabain		β-Adrenoceptor label (−)-[^{3}H]Di-hydroalprenolol	
Fraction	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Homogenate	1440	1	460	1	700	1	91.5	1	109	1	12.9	1
3500 × *g* pellet	2557	1.8	831	1.8	1149	1.64	144.4	1.6	364	3.3	34.0	2.6
45000 × *g* pellet	10145	7.05	2800	6.1	5275	7.5	671	7.3	708	6.5	31	2.4
Sucrose gradient												
Fraction 1	12278	8.5	3564	7.7	7063	10.0	1099	12	759	6.9	76.8	5.9
Fraction 2	15971	11.0	3418	7.4	7352	10.5	1026	11.2	717	6.5	125	9.7
Fraction 3	5069	3.5	1394	3.03	2197	3.0	318	3.5	1011	9.28	93	7.2
Fraction 4	32500	22.5	7868	17.1	12973	18.0	2124	23.2	2079	19.1	189	14.6

See [5,7] for further details. The following radioligands were employed: [^{3}H]ouabain (49 nM), [^{3}H]nimodipine (2.9 nM), [^{3}H]PN 200-110 (4.8 nM), d-*cis*-[^{3}H]diltiazem (7.1 nM), (−)-[^{3}H]dihydroalprenolol (2.05 nM), (−)-[^{3}H]desmethoxyverapamil (83 Ci/mmol, 2.19 nM). Fraction 4 consists mainly of t-tubule membranes. abs, specific binding in fmol/mg protein; rel, relative specific binding with respect to homogenate (defined as 1.0)

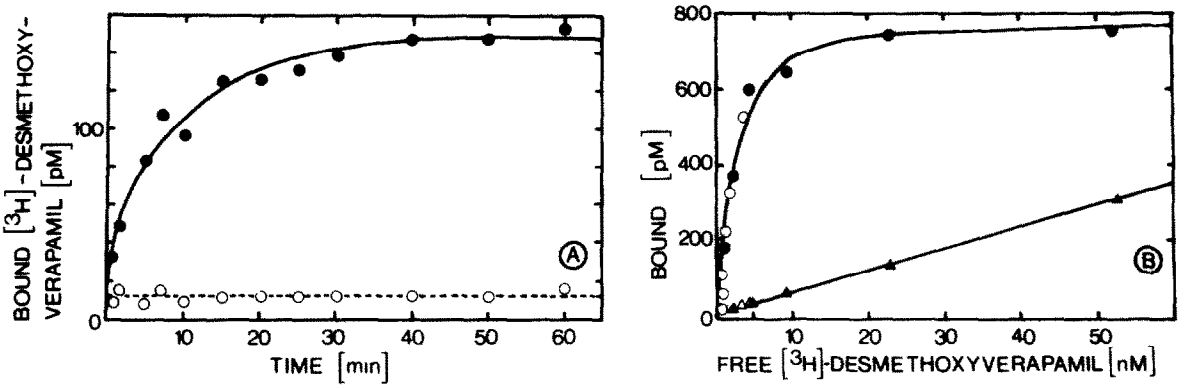

Fig.1. (A) Kinetics of (−)-[^{3}H]desmethoxyverapamil binding. Partially purified skeletal muscle membranes (4.4 μg in 0.25 ml) were incubated with 2.98 nM of (−)-[^{3}H]desmethoxyverapamil at the indicated times. Blank binding, defined by 10 μM (−)-D-600 (○), has been subtracted from total binding to yield specific binding (●). The line through the specific binding points is the best fit to the differential form of the second-order rate equation with the parameters (± asymptotic SD) k_{+1}, 0.054 ± 0.006 $nM^{-1} \cdot min^{-1}$ and k_{-1}, 0.032 ± 0.008 min^{-1}. (B) Saturation analysis of (−)-[^{3}H]desmethoxyverapamil. (−)-[^{3}H]Desmethoxyverapamil (83 Ci/mmol, ○, △ and 7.2 Ci/mmol, ●, ▲) was incubated with 13.7 μg skeletal muscle partially purified t-tubule membranes for 60 min at 25°C in a volume of 0.25 ml in the presence of 10 μM (−)-D-600 (△, ▲, blank binding). The line represents the best fit of the specific binding data to a monophasic saturation isotherm with a K_D of 2.1 ± 0.18 nM and B_{max} of 793 ± 23 pM (equivalent to 14470 ± 420 fmol/mg protein) with a slope factor of 1.08 ± 0.10.

[^{3}H]desmethoxyverapamil labelled sites in hippocampus membranes [10].

3.4. *Target size analysis*

Radiation inactivation experiments of (−)-[^{3}H]desmethoxyverapamil drug receptor sites were

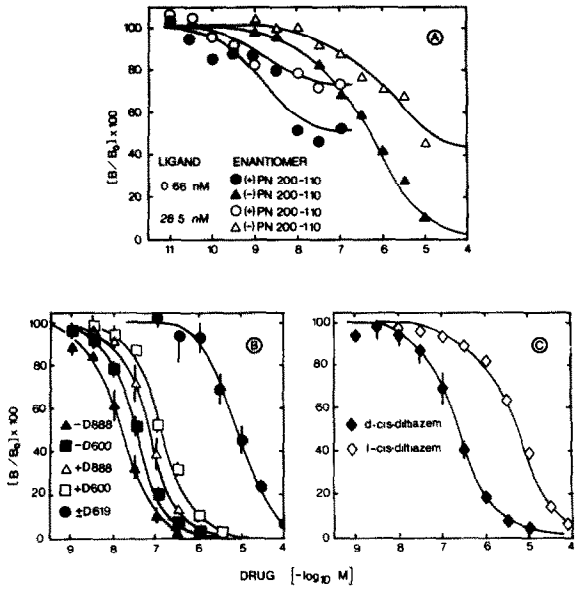

Fig.2. Binding inhibition studies. B_o is the specially bound ligand in the absence, and B in the presence of unlabelled drug. IC_{50} (or K_i) values and apparent Hill slopes (n_H) are given ± asymptotic standard deviation. For the competitive inhibitors, K_i values were calculated taking into account receptor concentration. (A) 1,4-Dihydropyridines. Binding inhibition studies were performed at two different ligand concentrations as indicated. At 0.66 nM, ^{3}H-ligand 53% of high affinity binding is inhibited by a saturating concentration of (+)-PN 200-110, but only 25%at 26.5 nM. The IC_{50} for the inhibitable binding is 2–3 nM at both ligand concentrations. At 0.66 nM, (−)-[^{3}H]desmethoxyverapamil ⩾ 90% of high affinity binding is inhibited by 10 μM (−)-PN 200-110, but only 50% at 26.5 nM, although the IC_{50} of (−)-PN 200-110 is ~500 nM in both cases. (B) Phenylalkylamines. (−)-D-600: K_i = 12 ± 1 nM, n_H = 1.22 ± 0.12; (+)-D-600: K_i = 55 ± 8 nM, n_H = 1.12 ± 0.16; (−)-desmethoxyverapamil: K_i = 3 ± 1 nM, n_H = 1.1 ± 0.2; (+)-desmethoxyverapamil: K_i = 17 ± 0.9 nM, n_H = 0.9 ± 0.1; (±)-D-619: K_i = 2855 ± 920 nM, n_H = 0.84 ± 0.18. (C) Benzothiazepines. d-*cis*-diltiazem: IC_{50} = 215 ± 23 nM, n_H = 1.11 ± 0.12; l-*cis*-diltiazem: IC_{50} = 6900 ± 600 nM, n_H = 0.93 ± 0.06.

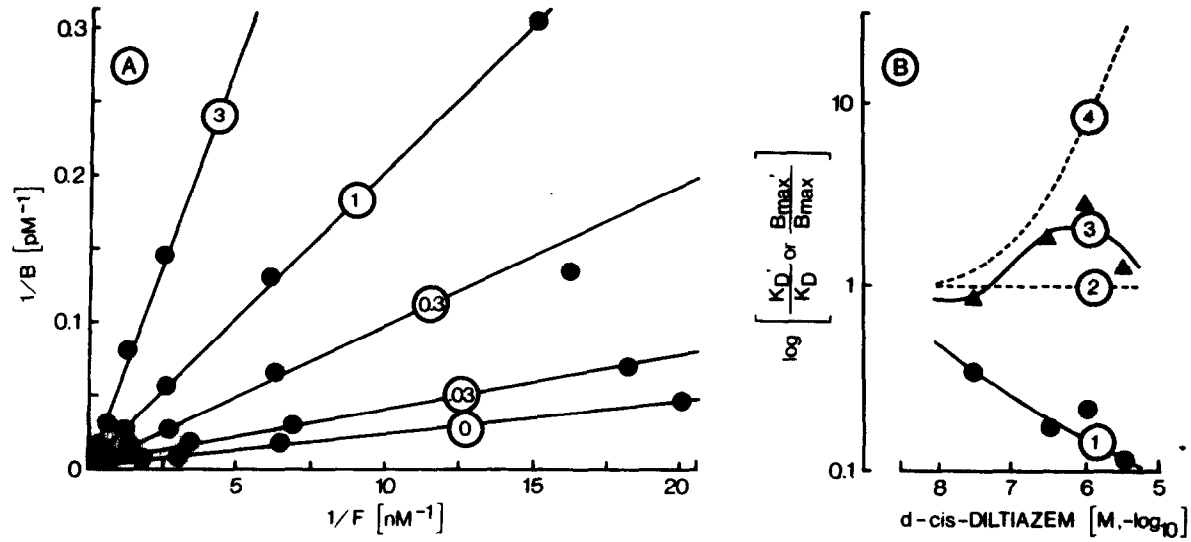

Fig.3. Nature of d-*cis*-diltiazem inhibition. (A) Saturation experiments were performed with (−)-[^{3}H]desmethoxyverapamil in the absence and presence of d-*cis*-diltiazem with the concentrations indicated. Primary reciprocal plots were linear (correlation coefficients > 0.95). (B) Apparent K_D' and apparent B_{max}' values of the radioligand in the presence of d-*cis*-diltiazem were normalized with respect to control K_D and B_{max} values. An apparent K_i for d-*cis*-diltiazem was calculated as follows: $I[\alpha/(1-\alpha)]$ was plotted against hot ligand concentration (I is the concentration of d-*cis*-diltiazem and α the ratio of bound hot ligand in the presence of the inhibitor to that in its absence) and extrapolated by linear regression to hot ligand = 0. The K_i was 153 nM. (1) Measured data for B_{max} ratio; (2) ratio of B_{max}'/B_{max} in the theoretical case of competitive inhibition; (3) measured ratio of K_D'/K_D; (4) calculated ratios of K_D'/K_D for K_i = 153 nM using $K_D'/K_D = 1/K_i + 1$, for the theoretical case of competitive inhibition.

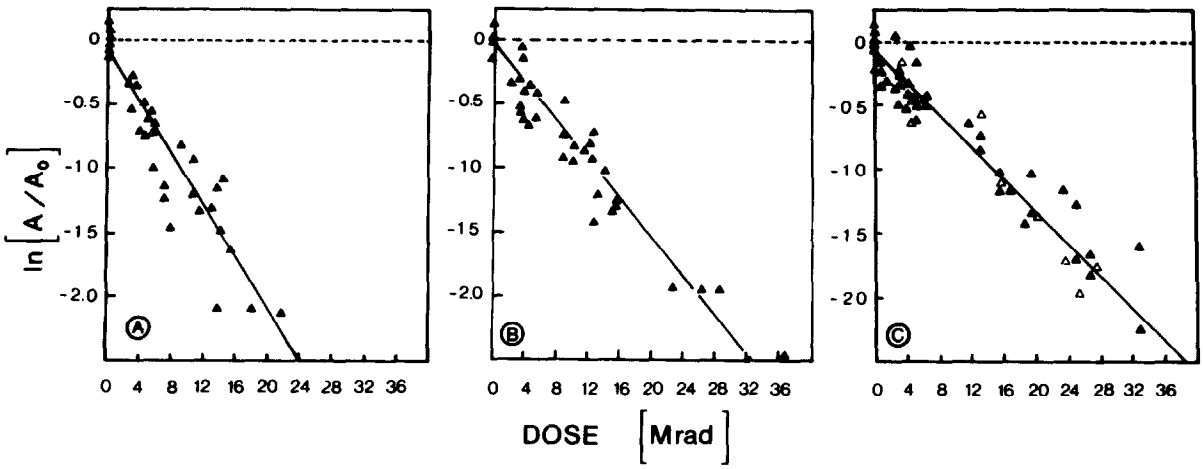

Fig.4. Radiation inactivation of the 3 drug receptor sites of the Ca^{2+}-channel. Data is pooled from 3–5 independent irradiations. The drawn lines have been calculated by linear regression analysis. (A) The decay of [^{3}H]nimodipine binding sites (179 ± 8 kDa). (B) The decay of d-*cis*-[^{3}H]diltiazem binding sites (130.5 ± 4 kDa). (C) The decay of (−)-[^{3}H]desmethoxyverapamil labelled sites (▲) (107 ± 2 kDa), and the decay of (±)-verapamil labelled sites in one experiment performed in parallel with one of the experiments contributing to the (−)-[^{3}H]desmethoxyverapamil data (△).

performed with partially purified t-tubule membranes. In parallel the target sizes of the benzothiazepine- and 1,4-dihydropyridine-selective sites were measured. Endogenous acetylcholinesterase activity was used as an internal control. The molecular mass of the acetylcholinesterase was 72 ± 3 kDa (not shown), in excellent agreement with our previous studies [7,13,14]. That of the (−)-[^{3}H]desmethoxyverapamil labelled site (fig.4) was 107 ± 2 kDa (n = 3), which is smaller than that of the d-*cis*-diltiazem-selective binding site (130.5 ± 4 kDa, n = 3, $p < 0.02$) or the 1,4-dihydropyridine site, labelled with [^{3}H]nimodipine. In one experiment the inactivation of the phenylalkylamine drug receptor site, labelled with (±)-[^{3}H]verapamil, was evaluated in the same samples. The molecular mass was found to be 109 kDa, in excellent agreement with what we reported in [6] and clearly identical to that of the (−)-[^{3}H]desmethoxyverapamil labelled site.

4. DISCUSSION

(−)-[^{3}H]Desmethoxyverapamil is an extremely useful radioligand to study phenylalkylamine selective drug receptor sites of the putative Ca^{2+} channel not only in brain [10] but also in skeletal muscle (here). (−)-[^{3}H]Desmethoxyverapamil is an optically pure enantiomer and has a 20-times lower K_D than racemic [^{3}H]verapamil. Unlike (±)-[^{3}H]verapamil [7], (−)-[^{3}H]desmethoxyverapamil binding can be studied with a good signal to noise ratio at 25°C where equilibrium is rapidly reached. The stereoselective inhibition of (−)-[^{3}H]desmethoxyverapamil binding by D-600 enantiomers correlates well with the stereoselectivity to block K^+-induced contractions of skeletal muscle [18]. The biologically weakly active phenylalkylamine (±)-D-619 [19] is also of low affinity. Further, d-*cis*-diltiazem is of higher potency than l-*cis*-diltiazem to inhibit (−)-[^{3}H]desmethoxyverapamil binding correlating with the potencies of the two diastereoisomers in functional Ca^{2+} antagonistic tests [17].

The subcellular fractionation experiments show that the (−)-[^{3}H]desmethoxyverapamil receptor sites are localized in the t-tubule where D-600, nifedipine [1] and d-*cis*-diltiazem [2] blockable Ca^{2+}-currents have been recorded. It has been proposed that Ca^{2+} channels in brain [10,20] skeletal muscle [3,7] and in heart [21] have 3 distinct, reciprocally allosterically coupled drug receptor sites. In skeletal muscle and brain [10] we find that d-*cis*-diltiazem and (+)-PN 200-110 act by binding t sites distinct from the phenylalkylamine drug receptor site labelled by (−)-[^{3}H]desmethoxyverapamil. The mode of binding-inhibition argues against the hypothesis [22] that the channel has only two receptor sites (one for the 1,4-dihydropyridines and one where d-*cis*-diltiazem and phenylalkylamines bind competitively).

The concept of 3 distinct drug receptor sites of the Ca^{2+} channel is also supported at a structural level by the radiation inactivation experiments. Thus, the molecular mass of the phenylalkylamine selective site is 110 kDa with (±)-[^{3}H]verapamil [7] or 107 kDa with (−)-[^{3}H]desmethoxyverapamil, despite the fact that the latter ligand labelled only 50% of the sites seen by the racemic ligand. These molecular masses are smaller than those of the benzothiazepine (130.5 kDa) or 1,4-dihydropyridine (179 kDa, labelled with [^{3}H]nimodipine) selective sites of the Ca^{2+}-channel. Our finding that the distinct drug receptor sites of the Ca^{2+}-channel have different target size analysis molecular masses is analogous to the results reported for the benzodiazepine receptor–GABA receptor–chloride ionophore complex in radiation inactivation experiments: A molecular mass of 54 kDa for the flunitrazepam-labelled subunit has been reported for the benzodiazepine receptor, but 135 kDa was found for the allosterically coupled site labelled with *t*-butylcyclophosphoro[^{35}S]thionate [23]. Also, it is interesting to note that the molecular mass of our d-*cis*-diltiazem-labelled drug receptor site is ~2- times larger than the decrease of ~60 kDa of the 1,4-dihydropyridine drug receptor induced by pre-incubation of membranes with d-*cis*-diltiazem [13,14]. This suggests that the channel component, dissociated by d-*cis*-diltiazem, may not be the drug receptor for d-*cis*-diltiazem. Recently it was shown (using Na^+/K^+-ATPase as an example [24]) that multifunctional oligomeric complexes when irradiated with 10 MeV electrons may exhibit 'radiation-sensitive domains' within a given polypeptide chain. This means that the molecular masses obtained for given binding sites (or enzymic function, etc.) may

in fact be smaller than those of the polypeptide on which they reside.

At the moment, however, the subunit composition of the Ca^{2+}-channel is not known with certainty. An arylazide 1,4-dihydropyridine, [^{3}H]azidopine [25] photoincorporates specifically into a polypeptide of 145 ± 5 kDa of guinea pig skeletal muscle t-tubule Ca^{2+}-channels. Further, a polypeptide of 130–160 kDa has been shown to copurify with pre-labelled [^{3}H]nitrendipine sites of rabbit skeletal muscle [26] (in addition to two smaller polypeptides of ~55 and ~30 kDa). A satisfactory interpretation of our target size analysis results can only be given when the channel has been conclusively purified to homogeneity and the subunits to which the chemically distinct groups of Ca^{2+} antagonists bind have been separated and individually identified by selective photoaffinity labelling. Nevertheless, the results obtained so far with target size analysis point to an oligomeric channel structure and underline fundamental differences in the way the Ca^{2+}-channel interacts with 1,4-dihydropyridine, phenylalkylamine and benzothiazepine Ca^{2+}-channel blocking agents.

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REFERENCES

[1] Stefani, E. and Chiarandini, D.J. (1982) Annu. Rev. Physiol. 44, 357–372.
[2] Gonzales-Serratos, H., Aguilera, R., Lathrop, D.A. and Garcia, M. del C. (1982) Nature 298, 292–294.
[3] Ferry, D.R. and Glossmann, H. (1982) FEBS Lett. 148, 331–337.
[4] Glossmann, H., Linn, T., Rombusch, M. and Ferry, D.R. (1983) FEBS Lett. 100, 226–232.
[5] Glossmann, H., Ferry, D.R. and Boschek, C.B. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 1–11.
[6] Fosset, M., Jaimovich, E., Delpont, E. and Lazdunski, M. (1983) J. Biol. Chem. 258, 6086–6092.
[7] Goll, A., Ferry, D.R. and Glossmann, H. (1984) Eur. J. Biochem. 141, 177–186.
[8] Galizzi, J.P., Fosset, M. and Lazdunski, M. (1984) Biochem. Biophys. Res. Commun. 118, 239–245.
[9] Nawrath, H. and Raschack, M. (1984) Cell Calcium 5, 316–317.
[10] Ferry, D.R., Goll, A., Gadow, C. and Glossmann, H. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 183–187.
[11] DeLean, A., Munson, P.J. and Rodbard, D. (1978) Am. J. Physiol. 4, E97–E102.
[12] Ferry, D.R., Goll, A. and Glossmann, H. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 292–297.
[13] Goll, A., Ferry, D.R. and Glossmann, H. (1983) FEBS Lett. 157, 63–69.
[14] Ferry, D.R., Goll, A. and Glossmann, H. (1983) EMBO J. 2, 1729–1732.
[15] Kepner, G.R. and Macey, R.I. (1968) Biochim. Biophys. Acta 163, 188–203.
[16] Kempner, E.S. and Haigler, H.T. (1982) J. Biol. Chem. 257, 13297–13299.
[17] Nagao, T., Sato, M., Iwasawa, Y., Takada, T., Ishida, R., Nakajima, H. and Kiyomoto, A. (1972) Jap. J. Pharmacol. 22, 467–478.
[18] Kaumann, A.J. and Uchitel, O.D. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 292, 21–27.
[19] Mannhold, R., Steiner, R., Haas, W. and Kaufmann, R. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 302, 217–226.
[20] Ferry, D.R. and Glossmann, H. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 80–83.
[21] Glossmann, H. and Ferry, D.R. (1982) Drug Dev. 9, 43–98.
[22] Murphy, K.M.M., Gould, R.J. and Synder, S.H. (1983) Proc. Natl. Acad. Sci. USA 45, 1560–1567.
[23] Nielsen, M. and Braestrup, C. (1983) Eur. J. Pharmacol. 96, 321–322.
[24] Ottolenghie, P. and Ellory, J.C. (1983) J. Biol. Chem. 258, 14895–14907.
[25] Ferry, D.R., Rombusch, M., Goll, A. and Glossmann, H. (1984) FEBS Lett. 168, 42–52.
[26] Curtis, B.M. and Catterall, W. (1984) Biochemistry 23, 2113–2118.