Temperature-dependent regulation of d-*cis*-[^{3}H]diltiazem binding to Ca^{2+} channels by 1,4-dihydropyridine channel agonists and antagonists

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The binding of the Ca^{2+}-channel blocker d-*cis*-[^{3}H]diltiazem to guinea pig skeletal muscle microsomes is temperature-dependent. At 2°C the K_D is 39 nM and B_{max} is 11 pmol/mg protein. The binding is fully reversible ($K_{-1} = 0.02\ min^{-1}$). The binding sites discriminate between the diastereoisomers l- and d-*cis*-diltiazem, reconize verapamil, gallopamil and tiapamil, and are sensitive to La^{3+}-inhibition. At 30°C the K_D is 37 nM and the B_{max} is 2.9 pmol/mg protein. D-*cis*-diltiazem-labelling is regulated by the 1,4-dihydropyridine Ca^{2+}-channel blockers and a novel Ca^{2+}-channel activator in a temperature-dependent manner. At 30°C an enhancement of d-*cis*-diltiazem binding by the channel blockers is observed. This is attributed to a B_{max} increase. EC_{50}-values for enhancement and the maximal enhancement differ for the individual 1,4-dihydropyridines. At 2°C 1,4-dihydropyridines inhibit d-*cis*-[^{3}H]diltiazem binding. This is attributed to a B_{max} decrease. We have directly labelled one of the drug receptor sites within the Ca^{2+}-channel which can allosterically interact with the 1,4-dihydropyridine binding sites.

d-cis-*[^{3}H]--diltiazem* *Skeletal muscle* *Ca^{2+}-channel* *1,4-Dihydropyridine*

1. INTRODUCTION

Radiolabelled 1,4-dihydropyridines can be employed to directly identify putative Ca^{2+}-channels in skeletal muscle membranes [1–3]. These skeletal muscle 1,4-dihydropyridine binding sites have a density which exceeds that of any other known source and can be enriched in t-tubular membranes [1,2]. The structurally unrelated Ca^{2+}-channel blocker d-*cis*-diltiazem acts as a positive allosteric regulator of 1,4-dihydropyridine binding [1]. This allosteric interaction with the 1,4-dihydropyridine site is not shared by the diastereoisomer l-*cis*-diltiazem, which is devoid of Ca^{2+}-channel blocking activity [4] and is a tissue- but not species-specific phenomenon [5].

Using guinea pig skeletal muscle microsomes as a Ca^{2+}-channel source and d-*cis*-[^{3}H]diltiazem we have directly labelled the drug receptor site within the putative Ca^{2+}-channel which mediates the allosteric regulation of 1,4-dihydropyridine binding.

2. MATERIALS AND METHODS

The sources and the specific activities of tritiated ligands were as follows: [^{3}H]nitrendipine (72 Ci/mmol) and [^{3}H]nimodipine (160 Ci/mmol) were from Bayer AG (Wuppertal); d-*cis*[*O-methyl*-^{3}H]-diltiazem (spec. act. 85 Ci/mmol) was supplied by Goedecke AG (Freiburg). Thin-layer chromatography on Kieselgel F_{254} (Merck, Darmstadt) with the solvent $CHCl_3/C_2H_5OH/H_2O/CH_3COOH$ (30:48:12:4, by vol.) demonstrated a radiochemical purity of >95%. Sources for unlabelled drugs have been given in [1,6]. Sea-anemone toxin II and tetrodotoxin were gifts from Professor Habermann (Giessen); other sodium channel toxins and drugs were from Dr Honerjäger (München). The 1,4-

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dihydropyridine Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) [7] as well as the nitrendipine derivative M 5579 were from Bayer AG (Wuppertal). Enantiomers of PN 200-110 (isopropyl 4-(2,1,3,benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycarboxy-pyridine-3-carboxylate) and the inactive 7-bromo substituted racemic PN 200-110 (Vo 2605) were from Sandoz AG (Basel). KG-944 (diethyl 4(benzothiazol-2-yl)-benzylphosphonate) was from Kanebo (Osaka).

Binding assays (filtration method) and preparation of guinea pig hind limb skeletal muscle microsomes were for labelled 1,4-dihydropyridines as in [1]. The d-*cis*-[^{3}H]diltiazem binding assays were performed in 50 mM Tris–HCl (pH 7.4) as described for the tritiated 1,4-dihydropyridines; saturable binding was defined as the difference between total radioactivity bound and that observed in the presence of 10 μM unlabelled d-*cis*-diltiazem. The assay volume was 0.25 ml, the incubation times and the temperature varied as outlined in section 3.

In brief, for the 2°C experiments 4 h of incubation with d-*cis*-[^{3}H]diltiazem at 0.5–400 nM was used. For evaluation of the pharmacological profile 3.5–7 nM d-*cis*-[^{3}H]diltiazem and 0.1–0.3 mg/ml of guinea pig skeletal muscle microsomal membrane protein was employed. Specific binding of d-*cis*-[^{3}H]diltiazem was linearly proportional to guinea pig microsomal protein (up to 0.6 mg/ml). At 0.1–0.3 mg protein/ml and 3.5–7 nM ^{3}H-ligand, 0.2–0.3% of the total radioactivity present in the assay was non-specifically trapped by Whatman GF/C filters. These blank values were close or identical to the filter blank (measured in the absence of membranes). The following drugs gave non-specific binding values indistinguishable from 10 μM d-*cis*-diltiazem: (–)D-600 (at 0.3 μM), (+)verapamil (at 5 μM) and tiapamil or KB 944 (at 10 μM). Equilibrium saturation isotherms at 2°C (4 h incubation) and 30°C (1 h incubation) were calculated from experiments in which the concentration of free radioligand was varied over a 60–800 range, by decreasing the specific activity and by changing the concentration of the radioligand, respectively. Data analysis for binding-inhibition experiments was as in [1].

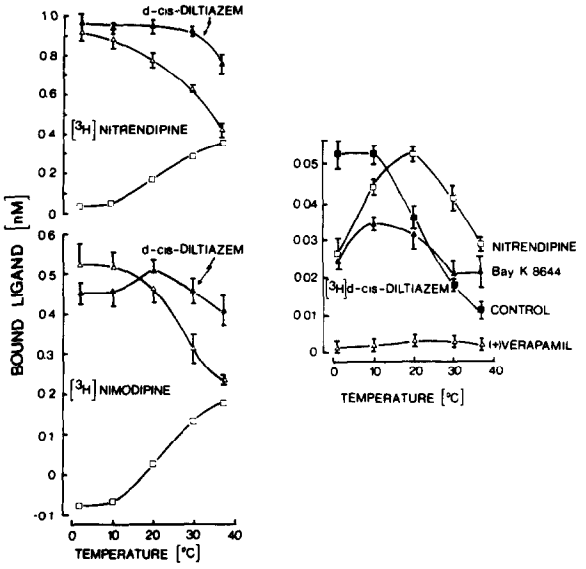

Fig.1. Temperature-dependent binding of [^{3}H]nitrendipine (4.43 nM), [^{3}H]nimodipine (1.56 nM) and d-*cis*-[^{3}H]diltiazem (3.12 nM) to guinea pig skeletal muscle microsomes (0.16 mg protein/ml). Each point is the mean of 3 expt ±SEM. In the case where [^{3}H]nitrendipine and [^{3}H]nimodipine labelled the Ca^{2+} channel, d-*cis*-diltiazem (10 μM) (closed symbols) was also present during the incubation; in the case were d-*cis*-[^{3}H]-diltiazem labelled the channel, nitrendipine (0.1 μM), Bay K 8644 (1 μM) and (+)verapamil (1 μM) were present during the incubation as indicated. The incubation times for the various temperatures were as follows: 2°C (4 h), 10°C (3 h), 20°C (2 h), 30°C (1 h), 37°C (30 min). For the 1,4-dihydropyridine labelling experiments of Ca^{2+} channels the calculated difference between the concentrations of ligand specifically bound in the absence and presence of d-*cis*-diltiazem is plotted as (□---□).

3. RESULTS

3.1. *Temperature dependence of d-cis-[^{3}H]diltiazem binding and of the d-cis-diltiazem effect on 1,4-dihydropyridine labelling of putative Ca^{2+}-channels in skeletal muscle microsomes*

Fig.1 shows an experiment where two tritiated chiral 1,4-dihydropyridines (nitrendipine and nimodipine) were employed to label the putative Ca^{2+} channel in guinea pig skeletal muscle microsomal membranes. The experiment was performed in the absence and presence of 10 μM d-*cis*-diltiazem. It can be seen that the stimulatory d-*cis*-diltiazem effect is highly temperature-dependent.

The specific binding of d-*cis*-[^{3}H]diltiazem was measured in parallel. In analogy to the experiment

with the labelled 1,4-dihydropyridines, where d-*cis*-diltiazem was present, several unlabelled 1,4-dihydropyridines were added at the indicated concentrations. The binding-temperature profile shows that an increase from 2–37°C leads to a 5-fold decrease in specific binding of d-*cis*-[^{3}H]diltiazem. However, this binding-temperature profile was characteristically changed by the simultaneous presence of 1,4-dihydropyridines. At 0°C and 10°C all 1,4-dihydropyridines including nimodipine (not shown), nitrendipine, PN 200-100 (not shown) and Bay K 8644 were inhibitory, whereas at ⩾20°C they were stimulatory to a different extent. As will be shown below, these stimulatory effects are stereospecific for chiral 1,4-dihydropyridines. The most interesting finding is the differential behaviour of the various 1,4-dihydropyridines. Included in this series is the novel 1,4-dihydropyridine, Bay K 8644, which activates instead of blocking Ca^{2+}-channels in guinea pig hearts and rabbit aortic strips [7] apparently by binding to the same drug receptor site as the 1,4-dihydropyridine channel blockers. Bay K 8644 had a K_i-value of 50 ± 15 nM for the [^{3}H]nimodipine binding site in skeletal muscle microsomal membranes (not shown).

This agonistic 1,4-dihydropyridine was of high efficacy to inhibit d-*cis*-[^{3}H]diltiazem binding at low temperatures, but was of low efficacy for stimulation at higher temperatures (see below). In contrast to these 1,4-dihydropyridines, (+) and (−)verapamil were inhibitoy at all temperatures.

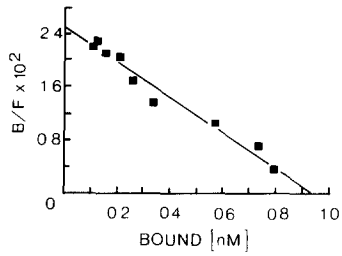

Fig.2. Equilibrium saturation isotherm of d-*cis*-[^{3}H]diltiazem binding to Ca^{2+}-channels at 2°C. Each point is the mean from duplicate determinations for both, total and non-specific binding; the data for specific binding are presented as a Scatchard plot. The concentration of guinea pig skeletal protein was 0.082 mg/ml. The correlation coefficient for the linear regression was 0.98; B_{max} = 0.94 nM (=11.465 fmol/mg protein); K_D = 37.5 nM.

3.2. *Equilibrium binding parameters, kinetics and pharmacological profile of the* d-cis-*[^{3}H]diltiazem binding sites*

The above experiments demonstrated that saturable d-*cis*-[^{3}H]diltiazem binding was dependent on the temperature and that this temperature depen-

Table 1

Equilibrium binding parameters and kinetic constants for d-*cis*-[^{3}H]diltiazem labelled Ca^{2+}-channels in guinea pig skeletal muscle microsomes

Parameter	Temperature							
	2°C Nitrendipine				30°C Nitrendipine			
	Absent		Present		Absent		Present	
B_{max} (fmol/mg protein)	11020 ± 1380	(6)[a]	6170 ± 1250	(3)[b]	2911 ± 608	(3)[c]	8400 ± 1400	(3)
K_d (nM)	39.0 ± 5.0	(6)	45 ± 7.6	(3)	37 ± 9	(3)	50 ± 7	(3)
Dissociation rate constant (min^{-1})	0.02 ± 0.01	(3)	n.d.		n.d.		n.d.	
Association rate constant ($nM^{-1}.min^{-1}$)	0.00053 ± 0.0001	(3)	n.d.		n.d.		n.d.	

Mean data ±SEM are given. The number of experiments are given in parentheses. Equilibrium binding parameters at 2°C and 30°C were also determined in the presence of 0.5 μM nitrendipine
n.d. = not determined; [a] $p < 0.05$ for 2°C *vs* 30°C; [b] $p < 0.05$ for nitrendipine present *vs* nitrendipine absent at 2°C; [c] $p < 0.05$ for nitrendipine absent *vs* nitrendipine present (Student's two-tailed *t*-test)

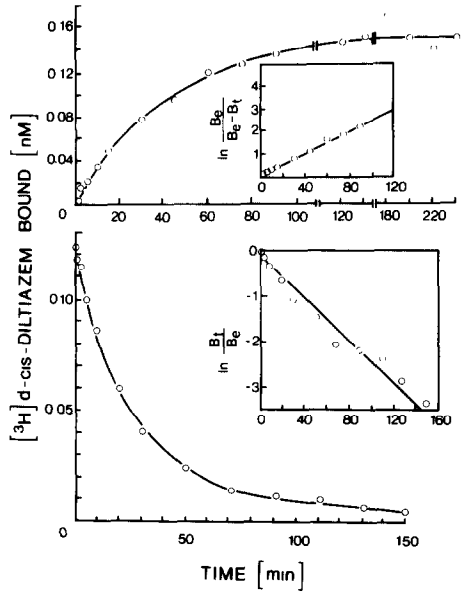

Fig.3. Association and dissociation kinetics of d-*cis*-[^{3}H]diltiazem. Upper panel: Guinea pig microsomal membranes (0.16 mg protein/ml) were incubated at 2°C with 7.35 nM d-*cis*-[^{3}H]diltiazem for the indicated times and the concentration of specifically bound ligand determined. Each point is the mean of 2 obs. for both total and non-specific binding (which did not increase with time). In the insert the kinetic data between 0 and 90 min are plotted according to the K_{obs} method. K_{obs} was 0.024 min^{-1} and k_{+1} (association rate constant) derived from K_{obs} and k_{-1} was 0.0059 $nM^{-1}.min^{-1}$. Direct non-linear curve-fitting of the data to the differential form of the second order rate equation yielded (± asymptotic standard deviation) 0.003 ± 0.0002 $nM^{-1}.min^{-1}$ for K_{+1} and 0.019 ± 0.0014 min^{-1} for the k_{-1}, and a receptor concentration of 1.6 nM. Lower: after 4 h of incubation of guinea pig skeletal muscle microsomal protein (0.072 mg/ml) at 2°C with 6.6 nM d-*cis*-[^{3}H]diltiazem (zero time point) the blockade of the forward reaction was initiated by addition of 10 μM d-*cis*-diltiazem. Each point is the mean of a duplicate experiment for the given times. Insert: Transformation of the dissociation data by plotting $\ln(B_t/B_e)$ yielded a slope of $-0.022\ min^{-1}$ (correlation coefficient: 0.97) $K_{-1} = 0.022\ min^{-1}$.

dence was opposed to the stimulatory effect of d-*cis*-[^{3}H]diltiazem on 1,4-dihydropyridine binding. It was found (fig.2, table 1) that at 2°C d-*cis*-diltiazem labelled 11 pmol/mg protein of binding sites, with a Hill coefficient being close to unity and a K_D of 39 nM, whereas at 30°C only 2.9 pmol/mg protein was labelled with the same K_D. Thus, at 30°C, a considerable fraction of the binding sites must be in a state which is either not available to the ligand or is of such low affinity ($K_D \geq 5\ \mu M$) that it cannot be measured with the filtration technology. The average kinetic parameters for d-*cis*-[^{3}H]diltiazem binding are given in table 1 and examples of kinetic experiments are shown in fig.3. Binding of d-*cis*-[^{3}H]diltiazem was fully reversible. The dissociation, induced by blockade of the forward reaction by unlabelled d-*cis*-diltiazem, was monophasic with a half-life of 35 min at 2°C. The K_D, derived from the rate constants (37.7 nM), is in excellent agreement with the K_d calculated from the equilibrium binding saturation isotherms. The pharmacological profiles of the d-*cis*-diltiazem binding sites are given in table 2. Most notable is, that the binding sites discriminate between l- and d-*cis*-diltiazem and recognize the structurally different Ca^{2+}-channel blockers verapamil, gallopamil, tiapamil and KB-944, which is an allosteric regulator of 1,4-dihydropyridine binding as is d-*cis*-diltiazem. The in-

Table 2

Pharmacological profile of the d-*cis*-[^{3}H]diltiazem binding sites determined at 2°C

Drug	IC_{50} (nM)	n_H
Ca^{2+}-Channel drugs:		
d-*cis*-Diltiazem	54.0 ± 1.4	0.99 ± 0.1
l-*cis*-Diltiazem	6680 ± 25.0	0.85 ± 0.04
(+)Gallopamil	42.1 ± 0.6	0.99 ± 0.06
(−)Gallopamil	17.5 ± 2.8	1.04 ± 0.04
(+)Verapamil	43.2 ± 0.6	1.02 ± 0.1
(−)Verapamil	54.0 ± 20.0	1.06 ± 0.14
Tiapamil	406.0 ± 45.0	1.19 ± 0.12
KB-944	358.0 ± 57.0	0.98 ± 0.03
La^{3+}	3.48 ± 0.82 × 10^5	1.09 ± 0.05
Na^+-Channel drugs:		
Tetrodotoxin	No effect	
Germitrine	No effect	
Sea anemone toxin II	No effect	
Veratridine	No effect	

n_H is the Hill slope and IC_{50} the concentration of drug causing 50% inhibition. IC_{50}-values and Hill slopes were derived from 3–4 binding-inhibition experiments where 7–9 concentrations of drug were tested in duplicate. Na^+ channel drugs were tested at concentrations between 1 and 100 μM

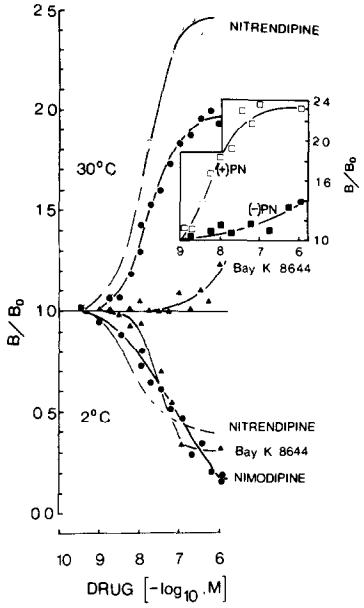

Fig.4. Temperature-dependent regulation of d-*cis*-[^{3}H]-diltiazem binding to guinea pig skeletal muscle microsomal membranes by 1,4-dihydropyridines. The concentration–effect curves were constructed by incubating 9–14 different concentrations of 1,4-dihydropyridines at either 2°C (lower), (4 h incubation) or 30°C (upper) with d-*cis*-[^{3}H]diltiazem and guinea pig skeletal muscle membranes. Each point is the mean from a duplicate determination for specific binding and the results are expressed as the ratio between B (concentration of d-*cis*-[^{3}H]diltiazem specifically bound in the presence of the respective 1,4-dihydropyridine) and B_0 (the specifically bound [^{3}H]ligand concentration in the absence of the 1,4-dihydropyridine). In the insert an experiment with enantiomers of PN 200-110 is shown: (+)PN = 205-033; (−)PN = 205-034.

organic Ca^{2+}- channel blocker La^{3+} also inhibited d-*cis*-[^{3}H]diltiazem binding, whereas several Na^+-channel drugs were completely inactive.

3.3. *Regulation of d-cis-[^{3}H]diltiazem binding by 1,4-dihydropyridines*

Depending on temperature, 1,4-dihydropyridines can either stimulate or inhibit the binding of d-*cis*-[^{3}H]diltiazem. This is in contrast to the enantiomers of verapamil, which were inhibitory at all temperatures investigated. Fig.4 shows the dose-dependency of the stimulatory effect of different 1,4-dihydropyridines at 30°C. It was found that the stimulation was stereospecific as the enantiomers of PN 200-110 display an eudismic ratio of $\geqslant$150 which is close to their eudismic ratio for the 1,4-dihydropyridine binding site labelled with [^{3}H]-nimodipine [1]. The individual 1,4-dihydropyridines differed with respect to the maximal stimulation and their EC_{50}-values. The respective parameters

Table 3

Effects of various 1,4-dihydropyridines on Ca^{2+}-channel labelling by d-*cis*-[^{3}H]diltiazem at 2°C and 30°C

Drug	2°C		30°C	
	IC_{50} (nM)	Maximal inhibition	EC_{50} (nM)	Maximal stimulation
Nitrendipine	8.75 ± 2.8	59.8 ± 1.9	33.5 ± 14	250 ± 4
Nimodipine	34.0 ± 14.7	83.3 ± 2.0	24.5 ± 6.4	200 ± 6
Bay K 8644	47.0 ± 1.2	64.0 ± 3.5	$\geqslant$500	123 ± 3
(+)205-033	7.8 ± 0.4	61.4 ± 0.4	6.1 ± 2.6	230 ± 14
(−)205-034	63.0 ± 11.0	81.0 ± 2.0	$\geqslant$1000	144 ± 7
Vo 2605	n.d.		no effect up to 1 μM	
M 5579	no effect up to 1 μM		no effect up to 1 μM	

For 2°C experiments the average maximal inhibition data as percentages $(B_0-B) \times 100$ (at $\leqslant 1\,\mu$M) and the average IC_{50}-values are given. For 30°C experiments the EC_{50}-values and the maximal stimulation values (at $\leqslant 1\,\mu$M) are presented. Stimulation is expressed as $(B/B_0) \times 100$; B is the concentration of d-*cis*-[^{3}H]diltiazem specifically bound in the presence of the 1,4-dihydropyridine and B_0 that in the absence of the 1,4-dihydropyridine, respectively. (+)205-033 is the (+) enantiomer of PN 200-110 and (−)205-034 is the (−)enantiomer. Vo 2506 is the 7-bromo-substituted PN 200-110 and M 5579 an inactive nitrendipine derivative with a free carboxyl group. Data are means of 2, or means of 3–4 (±SEM) separate experiments; n.d. = not determined

are given in table 3. From the 1,4-dihydropyridines investigated, nitrendipine and (+)enantiomer of PN 200-110 had the highest efficacy for stimulation. Most interesting is that the agonistic 1,4-dihydropyridine, Bay K 8644, was extremely weak with respect to stimulation. The benzoxadiazol 1,4-dihydropyridine Vo 2605, which is a 7-bromo substituted PN 200-110, and about 3 orders of magnitude weaker in affinity for the 1,4-dihydropyridine binding site as compared to PN 200-110 [8] was inactive, as was M 5579, which is an inactive nitrendipine derivative with a free carboxyl group [9]. To determine the nature of the d-*cis*-[^{3}H]diltiazem stimulation we have investigated the effects of nitrendipine (at 0.5 μM) on the equilibrium binding parameters at 30°C (table 1). The 1,4-dihydropyridine channel blocker increased the density of sites labelled by d-*cis*-[^{3}H]diltiazem at 30°C.

Fig.4 shows that the 1,4-dihydropyridines inhibited d-*cis*-[^{3}H]diltiazem binding at 2°C in a concentration-dependent manner. The respective mean IC_{50}-values and maximal inhibition percentages are also shown in table 3. The effects of 0.5 μM nitrendipine were tested on the equilibrium binding parameters as above. This 1,4-dihydropyridine channel blocker decreased the density of sites labelled by d-*cis*-[^{3}H]diltiazem at 2°C (table 1).

4. DISCUSSION

Our experiments show that d-*cis*-[^{3}H]diltiazem binds in a reversible manner and with an equilibrium dissociation constant of 39 nM to guinea pig skeletal muscle membranes. The binding sites were stereoselective as l-*cis*-diltiazem was of much lower affinity than d-*cis*-diltiazem. Verapamil, pallopmil, tiapamil and the d-*cis*-diltiazem-like Ca^{2+}-channel blocker KB-944, as well as the inorganic Ca^{2+}-channel antagonist La^{3+}, were inhibitory at the d-*cis*-[^{3}H]diltiazem binding sites; in contrast, Na^+-channel drugs were inactive. The binding of d-*cis*-[^{3}H]diltiazem was temperature dependent and 1,4-dihydropyridines regulated the binding in a complex manner. At 30°C the 1,4-dihydropyridines, to a different extent, stimulated stereospecifically (the eudismic ratio of the two enantiomers of PN 200-110 was $\geqslant$150), whereas at 2°C they inhibited. In addition, two 1,4-dihydropyridine derivatives which are of extremely low affinity for the 1,4-dihydropyridine drug receptor and are almost devoid of Ca^{2+}-channel blocking activity, did not stimulate at all. We conclude, that we have directly labelled the receptors for Ca^{2+}-channel drugs, which act as positive heterotropic allosteric regulators on the 1,4-dihydropyridine binding sites. The stoichiometry between the allosteric sites and the 1,4-dihydropyridine binding sites appears to be 1:1 or 1:2, depending on the density of the latter, which is a function of the radioligand [10].

In the absence of 1,4-dihydropyridines, d-*cis*-[^{3}H]diltiazem labelled more sites at 2°C than at 30°C. It is therefore reasonable to conclude that the marked temperature-dependence of d-*cis*-[^{3}H]diltiazem binding reflects the temperature-dependence of the constant governing the equilibrium between high affinity (K_D 39 nM) and low affinity states ($K_D \geqslant 5\,\mu$M) of the Ca^{2+}-channel d-*cis*-diltiazem binding sites.

Likewise, the same conclusion may be drawn for the 1,4-dihydropyridine labelling of Ca^{2+}-channels, since we show that, as for d-*cis*-[^{3}H]diltiazem, maximal labelling occurs at $\leqslant$10°C. One of the actions of d-*cis*-diltiazem at $\geqslant$20°C is the establishment of a new equilibrium of low and high affinity states for 1,4-dihydropyridine channel blockers, favouring the high-affinity state [1,5,6,10]. In accord with this, the d-*cis*-diltiazem effect on the 1,4-dihydropyridine labelling of the channels was minimal or absent at 2°C and was maximal at 37°C. In view of the Ca^{2+}-channel blocking activity of d-*cis*-diltiazem and the 1,4-dihydropyridines nimodipine and nitrendipine, it is tempting to postulate that the high-affinity labelled channel for these radioligands is a 'shut' channel, whereas the low affinity state is equivalent to the 'unshut' channel. Whether or not for example the hypothetical 'shut' channel-state in depolarized membrane fragments is comparable to the open (but drug-blocked), closed or inactivated channel-states, as they may occur in intact tissues, is an open question. Based on the above assumptions we provide the following hypothesis for the differential temperature effect of the 1,4-dihydropyridines and their different efficacies for stimulation (e.g. at 30°C) in comparison to their efficacy for inhibition (at 2°C):

The 1,4-dihydropyridine Ca^{2+}-channel blockers increase the fraction of channels in 'shut' state for

d-*cis*-[^{3}H]diltiazem at 30°C; but, because their relative affinities for 'shut' and 'unshut' channels differ, they vary in their intrinsic efficacy. Nitrendipine > (+)205-033 > nimodipine > (−)205-034 > Bay K 8644 ⩾ Vo 2650, M 5579 is then the rank order of efficacies for the conversion into the 'shut' state for these 1,4-dihydropyridines.

In contrast, at 2°C, where all of the channels which can be labelled with d-*cis*-[^{3}H]diltiazem are 'shut' channels, the rank order of efficacies for keeping channels in the 'unshut' state was quite different: nimodipine = (−)205-034 > Bay K 8644 = (+)205-033 = nitrendipine. One must conclude that none of the 1,4-dihydropyridines investigated can be a pure Ca^{2+}-channel agonist or antagonist. This is indeed the case. Bay K 8644 is only a partial agonist [7] and nimodipine, when compared to nitrendipine in classical pharmacological experiments, has stronger agonistic properties (Towart, personal communication). Most interesting in this context is the behaviour of the enantiomers of the PN 200-110. One would expect, based on our hypothesis, that the (−)enantiomer, compared to the (+)enantiomer, may behave as a partial Ca^{2+}-channel agonist, whereas the (+)enantiomer could be an almost pure antagonist. Also, the eudismic ratio of the EC_{50}-values for the two enantiomers, which is ⩾150 for stimulation, was reduced to ≈10 when the IC_{50}-values for inhibition are compared. Clearly, the interaction of Ca^{2+}-channel drugs with their binding sites is of remarkable complexity and far from being understood. The concept of a continuum of Ca^{2+}-channel drugs ranging from agonists to antagonists was proposed earlier, based on a comparison of Ca^{2+}-channel labelling by different 1,4-dihydropyridines in skeletal muscle microsomes [10]. The present findings, with d-*cis*-[^{3}H]diltiazem labelling of the allosteric regulatory site, support this concept. One important conclusion is, that affinities of Ca^{2+}-channel drugs, found in classical pharmacological or electrophysiological experiments, may or may not be identical to the affinities measured by direct labelling of the channels. The partial agonism of the drugs, taken together with the possible existence of subtypes of Ca^{2+}-channels [5,8], may be the molecular basis for the apparent tissue selectivity of Ca^{2+}-channel drugs which is so far not understood but has considerable therapeutic implications.

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REFERENCES

[1] Ferry, D.R. and Glossmann, H. (1982) FEBS Lett. 148, 331–337.
[2] Fosset, M., Jaimovich, E., Delpont, E. and Lazdunski, M. (1983) Eur. J. Pharmacol. 86, 141–142.
[3] Fairhurst, A.S., Thayer, S.A., Colker, J.E. and Beatty, D.A. (1983) Life Sci. 32, 1331–1339.
[4] Nagao, T., Sato, M., Iwasawa, Y., Takada, T., Ishida, R., Nakajima, H. and Kiyomoto, A. (1972) Japan J. Pharmacol. 22, 467–478.
[5] Ferry, D.R. and Glossmann, H. (1983) Brit. J. Pharmacol. 78, 81P.
[6] Glossmann, H., Ferry, D.R. and Boschek, C.B. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 1–11.
[7] Schramm, M., Thomas, G., Towart, R. and Franckoniak, G. (1983) Nature 303, 535–537.
[8] Glossmann, H. and Ferry, D.R. (1983) Drug Dev. 9, 63–98.
[9] Glossmann, H., Ferry, D.R., Lübbecke, F., Mewes, R. and Hofmann, F. (1982) Trends Pharmacol. Sci. 3, 431–437.
[10] Ferry, D.R., Goll, A. and Glossmann, H. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 276–277.