Biochemical Pharmacology, Vol. 35, No. 10, pp. 1639–1646, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

DIFFERENCES IN Ca^{2+} ENTRY BLOCKERS REVEALED BY EFFECTS ON ADENOSINE- AND ADRENERGIC-RECEPTORS AND CYCLIC AMP LEVELS OF [2-^{3}H]ADENINE-PRELABELED VESICLES PREPARED FROM GUINEA PIG BRAIN

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(*Received* 23 *May* 1985; *accepted* 23 *September* 1985)

Abstract—Three major classes of Ca^{2+} entry blockers, classified according to effects on cardiac and vascular smooth muscle, were tested. Vesicles prepared from cerebral cortex and stimulated by adenosine and epinephrine constituted adenosine and alpha-adrenergic receptor systems respectively. Vesicles prepared from cerebellum and stimulated by epinephrine constituted the beta-adrenergic receptor system. Experiments with adenosine were also performed with vesicles formed or incubated in the absence of exogenous Ca^{2+}. The results indicate that Ca^{2+} entry blockers had a variety of effects, even within classes of drugs. Vascular-selective group A Ca^{2+} entry blockers such as nifedipine and nisoldipine antagonized adenosine, but the structurally-related drug nitrendipine was inactive. Inhibition was competitive with adenosine and independent of exogenous Ca^{2+}. In contrast to receptor-binding studies requiring high ratios of the drugs to adenosine receptor radioligands, nifedipine and nisoldipine were inhibitory at equimolar concentrations with adenosine. Non-selective group A Ca^{2+} entry blockers such as diltiazem and verapamil were inactive against adenosine. Group B Ca^{2+} entry blockers, prenylamine and perhexilene, increased cyclic AMP (cAMP) levels of vesicles stimulated by adenosine but not by epinephrine or under basal conditions. This effect was observed only in vesicles that had been formed in the presence of Ca^{2+}. Ca^{2+} entry blockers also exhibited effects on adrenergic receptors unrelated to effects on adenosine. Verapamil and prenylamine acted as alpha-adrenergic antagonists and only prenylamine acted as a beta-adrenergic antagonist. However, the vesicle system also revealed indirect blocking actions of nifedipine on adrenergic receptor systems. The actions of the Ca^{2+} entry blockers are discussed in relation to the special usefulness of nifedipine in the treatment of patients with defective atrioventricular conduction and also in relation to the unique ability of group B Ca^{2+} entry blockers to selectively inhibit Ca^{2+} and calmodulin activated phosphodiesterase. However, some caution must be applied in drawing conclusions relating to the cardiovascular actions of these drugs from data generated in a neuronally-derived model.

Ca^{2+} entry blockers may become the drugs of choice for most patients with angina [1]. They act principally by depressing contraction of cardiac and vascular smooth muscle. However, the drugs are neither structurally nor pharmacologically homogeneous and can be classified into three types: group A, vascular-selective group A, and group B [1]. Representatives of the three types are shown in Fig. 1.

There is evidence that Ca^{2+} entry blockers act on adrenergic and adenosine receptors, that they differ in this respect even within types classified by their action on muscle (above), and that some actions are not mediated by effects on Ca^{2+}. Verapamil binds to alpha-adrenergic receptors in rat liver membranes but does not influence Ca^{2+} movement into or out of the intact liver cell [2]. Verapamil and a nifedipine analog prevent high-affinity binding of the radioligand [^{3}H]WB-4101 to alpha-adrenergic receptors and prevent low-affinity binding of the radioligand to Ca^{2+} channel sites in rat brain membranes [3]. Verapamil binds to the alpha-adrenergic receptor in rabbit uterus but has no affinity for the beta-adrenergic receptor in guinea pig heart; perhexilene binds to the beta- but not to the alpha-adrenergic receptor [4].

Evidence for the interaction of Ca^{2+} entry blockers with the adenosine receptor is mixed. Nifedipine inhibits the coronary vasodilator action of adenosine *in vivo* [5] but does not antagonize adenosine in more indirect *in vitro* experiments [6, 7]. Receptor-binding studies show that nifedipine interacts with A_1- and A_2-adenosine receptors in rat brain membranes, but its action is believed to be indirect [8]. Other Ca^{2+} entry blockers such as verapamil and diltiazem are inactive [8].

The vesicle adenylate cyclase system [9] represents an alternative experimental method for studying the action of drugs on adenosine [10] and adrenergic [11] receptors. Vesicles are also useful for differentiating direct actions of drugs on receptors from those mediated by Ca^{2+} since vesicles can be formed and used in the presence or absence of Ca^{2+} [11]. Our principal objective was to test seven different Ca^{2+} entry blockers for possible differences in their actions on adrenergic and adenosine receptors as revealed by the use of vesicles.

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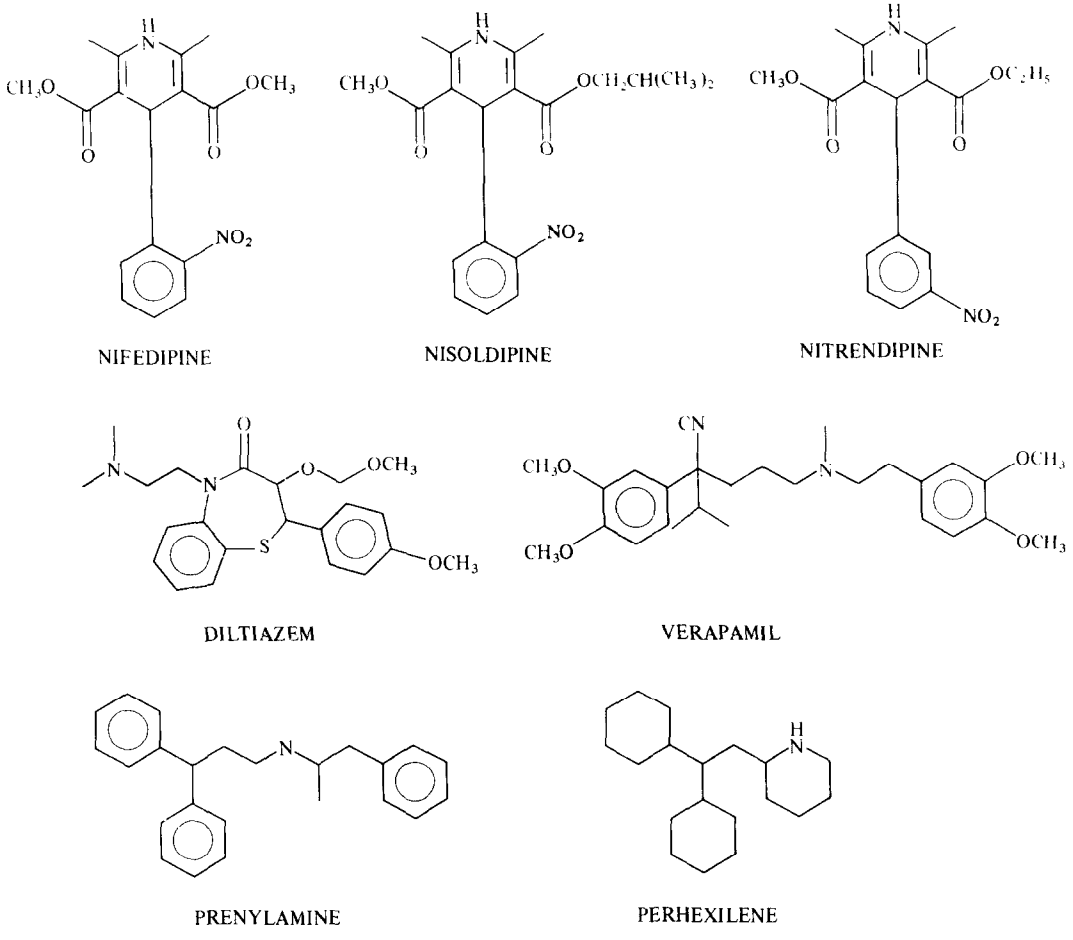

Fig. 1. Chemical structures of three types of Ca^{2+} entry blockers. Nifedipine, nisoldipine and nitrendipine are vascular-selective group A drugs. Diltiazem and verapamil are non-selective group A drugs. Prenylamine and perhexilene are group B drugs.

METHODS

Preparation and use of brain vesicles for adenylate cyclase experiments. For all experiments except those involving the beta-adrenergic receptor, one guinea pig was decapitated, and the brain was quickly removed, surrounded with ice, and allowed to cool for at least 10 min. For studies with the beta-adrenergic receptor, four to five guinea pigs were decapitated, and the cerebellums were quickly removed following each decapitation and surrounded with ice. The entire cerebral cortex, dissected out on ice (and including the hippocampus), or the cerebellums were homogenized with 10 ml of ice-cold Krebs–Ringer bicarbonate buffer [11] with three or four single downward strokes in an all-glass, motor-driven 15-ml Tenbroeck homogenizer; the motor speed ranged from 1100 to 1500 rpm. After each stroke, the motor was shut off and the pestle was removed by hand from the tube. The remaining tissue plug in the bottom of the tube was dislodged with a spatula in order to facilitate its homogenization during the following stroke. After the final stroke, the pestle was allowed to remain in the tube and the homogenate above the tube (about 10 ml) was decanted into a 15-ml conical graduated plastic tube and centrifuged at 1000 *g* for 15 min at 1–2° in a centrifuge equipped with a swing-out head. The supernatant fraction was decanted and discarded, and the white upper third of the sediment was removed by suction and discarded. Cold buffer (8 ml) was used to resuspend the remaining sediment and transfer it into a 50-ml plastic tube. Adenine (10 μl of a solution containing 0.11 mg/ml) and [2-^{3}H]adenine (120 μl of a solution containing 1 mCi/ml) were added, and the air space in the tube was gassed with O_2–CO_2 (95%–5%) for 30 sec. The tube was capped and incubated at 37° in a longitudinal horizontal position with shaking (36 cycles/min) for 20 min. Cold buffer was then added to bring the contents to 40 ml, and the tube was centrifuged under the same conditions as above. The supernatant fraction was discarded, and the sediment was first resuspended as described above and then brought to a final volume of 45 ml with cold buffer. In experiments where vesicles were prepared and labeled with [2-^{3}H]adenine in normal buffer and subsequently washed and used in Ca^{2+}-free buffer, an extra washing step with the Ca^{2+}-free buffer was included. Aliquots (1 ml) of brain vesicles were added to glass scintillation vials and equilibrated at 37° with shaking (104 cycles/min) for 40 min under O_2–CO_2.

Table 1. Basal and adenosine-stimulated cAMP levels of guinea pig cerebral cortex vesicles prepared and incubated under different conditions of Ca^{2+}

Ca^{2+} (2.6 mM)	N	Percent conversion of [2-^{3}H]adenine to [^{3}H]cAMP Basal	Adenosine (5 μM)	Percent increase by adenosine over basal
A. Present during vesicle preparation and incubation	12	0.39 ± 0.03 (8,311 ± 639 dpm) (2.49 ± 0.19 pmoles)	2.78 ± 0.24 (59,242 ± 5,114 dpm) (17.77 ± 1.53 pmoles)	605
B. Present during vesicle preparation only	12	0.39 ± 0.03 (6,595 ± 507 dpm) (1.98 ± 0.15 pmoles)	2.60 ± 0.23 (43,970 ± 3,889 dpm) (13.19 ± 1.17 pmoles)	574
C. Absent during vesicle preparation and incubation	3	0.23 ± 0.04 (3,997 ± 692 dpm) (1.19 ± 0.21 pmoles)	0.45 ± 0.15 (7,781 ± 2,593 dpm) (2.33 ± 0.78 pmoles)	96

Values are given as the mean ± S.E.M. per ml vesicles. [2-^{3}H]Adenine was taken up by vesicles, incorporated into endogeneous ATP, and converted to [^{3}H]cAMP. Percent conversion is a measure of intrinsic activity of vesicles and independent of numbers of vesicles used, extent of [2-^{3}H]adenine uptake, and specific activity of [2-^{3}H]adenine. Disintegrations per minute (dpm) and picomoles of cAMP have been calculated, the latter on the basis of the combined specific activity (1.5 Ci/mmole) of [2-^{3}H]adenine and non-radioactive adenine used.

Following the equilibration period, 0.5 ml of buffer solution alone, or the same solution containing the desired neurohormone and/or Ca^{2+} entry blocker at three times the final desired concentrations, was added to the 1-ml aliquots of vesicles, and incubation was continued with shaking at 37° under O_2–CO_2 for 15 min. Each vial then received 0.5 ml of trichloracetic acid (240 g/l) containing cyclic AMP (cAMP) (20 mg/l). Following centrifugation for 7 min at 1000 *g* in 15-ml centrifuge tubes, 1-ml aliquots of the supernatant fractions were assayed for [^{3}H]cAMP. Each 1-ml aliquot was passed through Dowex 50 W-X8 resin (5 × 33 mm) in glass columns followed by 2 ml of H_2O, and the effluents were discarded. H_2O (4 ml) was then added to the columns, and the major, central portion of cAMP was thus eluted. The 4-ml effluents were treated with 0.2 ml each of 0.25 M $Ba(OH)_2$ and 0.25 M $ZnSO_4$, centrifuged for 7 min at 1000 *g*, and the supernatant fractions treated once more with $Ba(OH)_2$ and $ZnSO_4$. A 2.5-ml aliquot of the final supernatant fraction was then counted in the presence of 17.5 ml of Hydrofluor scintillation solution.

Table 2. Inhibitory and stimulatory actions of Ca^{2+} entry blockers on adenosine-stimulated cAMP synthesis in vesicles from guinea pig cerebral cortex prepared and incubated under different conditions of Ca^{2+}

Ca^{2+} entry blocker	Medium Ca^{2+}	Change (%) in cAMP by Ca^{2+} entry blockers at various concentrations (μM): 1	3	10	Approx. IC_{50} or ED_{50} (μM)
Nifedipine	A	−14 ± 4†	−34 ± 6†	−69 ± 5†	5.2
	B	−8 ± 2	−39 ± 2†	−78 ± 5†	4.3
	C		−30 ± 3*	−62 ± 3*	6.4
Nisoldipine	A	−15 ± 3‡	−23 ± 2*	−60 ± 6†	7.2
	B	−18 ± 1*	−23 ± 1*	−72 ± 3†	5.9
	C		−26 ± 5†	−65 ± 5†	6.4
Prenylamine	A	−6 ± 4	11 ± 6	56 ± 6*	8.6
	B	−6 ± 3	5 ± 3	31 ± 6†	
	C			− 3 ± 12	
Perhexilene	A	−4 ± 3	15 ± 8‡	71 ± 31†	6.4
	B	−10 ± 5*	1 ± 6	48 ± 13†	10.1
	C			11 ± 15	

Conditions A, B and C are defined in Table 1. Adenosine was used at 4 μM to stimulate cAMP synthesis. Effects of drugs were calculated as the percent change of control for each experiment, after subtraction of the basal cAMP level. Values shown are the mean ± S.E.M. for three to four separate experiments, each performed in triplicate or quadruplicate. Mean values for all controls used in these experiments are given in Table 1. Statistical evaluation was as described in Methods. Nitrendipine, diltiazem and verapamil were inactive up to concentrations of 10, 100 and 30 μM respectively.

* $P < 0.01$.

† $P < 0.001$.

‡ $P < 0.05$.

Table 3. Basal and epinephrine-stimulated cAMP levels of vesicles prepared from guinea pig cerebral cortex (alpha-adrenergic) and guinea pig cerebellum (beta-adrenergic) vesicles

Source of vesicles	N	Percent conversion of [2-^{3}H]adenine to [^{3}H]cAMP Basal	Epinephrine (2 μM)	Percent increase by epinephrine over basal
Cerebral cortex	5	0.33 ± 0.03 (10,474 ± 838 dpm) (3.14 ± 0.25 pmoles)	0.99 ± 0.12 (31,873 ± 3,803 dpm) (9.56 ± 1.14 pmoles)	204
Cerebellum	6	0.34 ± 0.03 (6,528 ± 505 dpm) (1.96 ± 0.15 pmoles)	5.11 ± 0.54 (99,183 ± 10,550 dpm) (29.75 ± 3.17 pmoles)	1,419

Values are given as the mean ± S.E.M. per ml vesicles and calculated as described in Table 1.

Materials. Verapamil was donated by Knoll, A. G. (Ludwigshafen, West Germany). Other Ca^{2+} entry blockers were synthesized by CIBA-GEIGY chemists. The sources of other materials were described previously [11].

Statistics. Since the capacity of each experiment was limited to thirty-six vials, experiments were designed to accommodate various combinations of drugs resulting in many different combinations of controls. Drug action was thus calculated as the percent change from control of each experiment, after subtraction of the basal value, and the mean percent change of three to four separate experiments was determined. This was compared to the constant 0, and a one sample *t*-test was done when only one concentration of drug was tested. When more than one concentration of drug was tested, an analysis of variance was performed with a trend test [12]. Averages of all basal and control values for each of the three hormone receptor systems studied were also calculated (Tables 1 and 3).

RESULTS

Effects of Ca^{2+} *entry blockers on adenosine-stimulated [*^{3}*H]cAMP levels of vesicles*. Averages of control levels for vesicles prepared under three different conditions of Ca^{2+} (conditions A, B or C) are seen in Table 1. [^{3}H]cAMP levels expressed as percent conversion from [2-^{3}H]adenine were similar when vesicles were prepared in the presence of Ca^{2+} (conditions A and B) but reduced ($P < 0.05$, basal; $P < 0.001$, adenosine) when vesicles were prepared in Ca^{2+}-free buffer (condition C). A fourth possible condition, vesicles prepared in the absence of Ca^{2+} and incubated in its presence, was not used for testing Ca^{2+} entry blockers since Ca^{2+} did not restore the reduced level of [^{3}H]cAMP in these vesicles (data not shown). Table 2 shows the effects of Ca^{2+} entry blockers on adenosine-stimulated vesicles. Two of the three vascular-selective group A drugs tested, nifedipine and nisoldipine, were inhibitory. Inhibition by nifedipine was independent of the conditions of Ca^{2+} (Table 2) and was competitive with adenosine (Fig. 2). Both non-selective group A drugs, diltiazem and verapamil, were inactive. The two group B drugs, prenylamine and perhexiline, increased adenosine-stimulated [^{3}H]cAMP levels in vesicles that had been formed in the presence of Ca^{2+} (conditions A and B) but not in its absence (condition C). None of the drugs affected basal [^{3}H]cAMP levels.

Effects of Ca^{2+} *entry blockers on epinephrine-stimulated [*^{3}*H]cAMP levels in vesicles containing alpha-adrenergic receptors*. The average of control levels of [^{3}H]cAMP is shown in Table 3. The three vascular-selective group A drugs, nifedipine, nisoldipine and to a lesser extent nitrendipine, were inhibitory (Table 4). Inhibition by nifedipine was not competitive with epinephrine (Fig. 3). There was no uniform pattern of activity for the remaining groups of Ca^{2+} entry blockers. Verapamil was inhibitory,

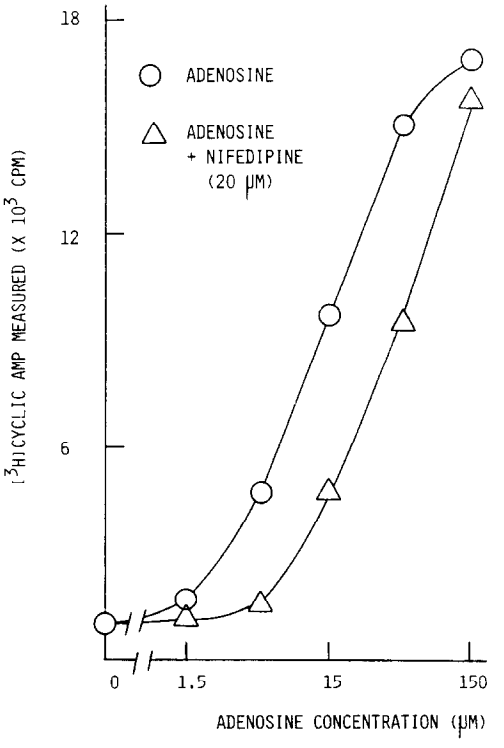

Fig. 2. Competitive inhibition by nifedipine of adenosine-stimulated cAMP formation. Vesicles were prepared from guinea pig cerebral cortex, prelabeled with [2-^{3}H]adenine, preincubated for 40 min, and then stimulated for 15 min with various concentrations of the agonist in the presence or absence of the Ca^{2+} entry blocker. Basal cAMP levels were not affected by the Ca^{2+} entry blockers studied. Each value is the mean of three to four replications in a typical experiment. Values are given directly as cpm in order to illustrate actual data measured; further calculation of data would not have altered the curves. Because of the excellent replication inherent in this experimental system, standard errors were small in individual experiments and not shown. Half-maximal activation by adenosine occurred at 13 μM.

Table 4. Inhibitory actions of Ca^{2+} entry blockers on epinephrine-stimulated cAMP synthesis via the alpha-adrenergic receptor in vesicles from guinea pig cerebral cortex

Ca^{2+} entry blocker	Change (%) in cAMP by Ca^{2+} entry blockers at concentrations (μM): 1	3	10	30	Approx. IC_{50} (μM)
Nifedipine	−31 ± 7*	−57 ± 2*	−85 ± 1*		2.2
Nisoldipine	−22 ± 1*	−33 ± 3*	−74 ± 6*		4.8
Nitrendipine		−1	−34 ± 7†		
Verapamil			−36 ± 4*	−63 ± 2*	18.
Prenylamine		−38 ± 3*	−53 ± 2*		8.7

Vesicles were prepared and incubated in the presence of Ca^{2+}. Epinephrine was used at 2 μM to stimulate cAMP synthesis. Mean values for all controls used in these experiments are given in Table 3. Calculations were as given in Table 3. Diltiazem and perhexilene were inactive up to 100 and 10 μM respectively.

* $P < 0.001$.

† $P < 0.05$.

but diltiazem was inactive (Table 4). In contrast to its activating action in the presence of adenosine (Table 2), prenylamine was inhibitory against epinephrine (Table 4) and competitive (Fig. 4). Perhexilene was inactive.

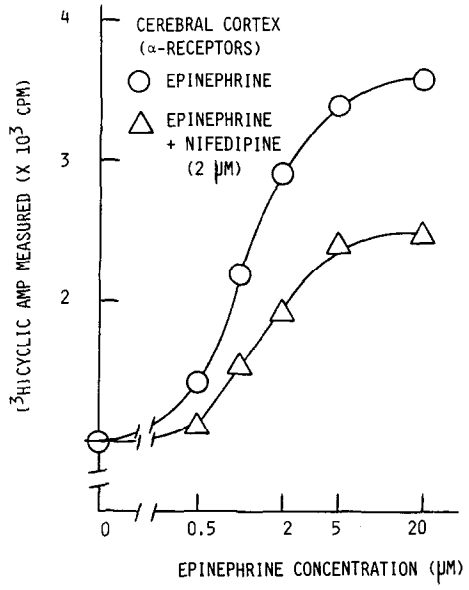

Fig. 3. Non-competitive inhibition by nifedipine of epinephrine-stimulated cAMP formation via alpha-adrenergic receptors. General conditions were as described in the legend of Fig. 2. Half-maximal activation by epinephrine occurred at 1.1 μM.

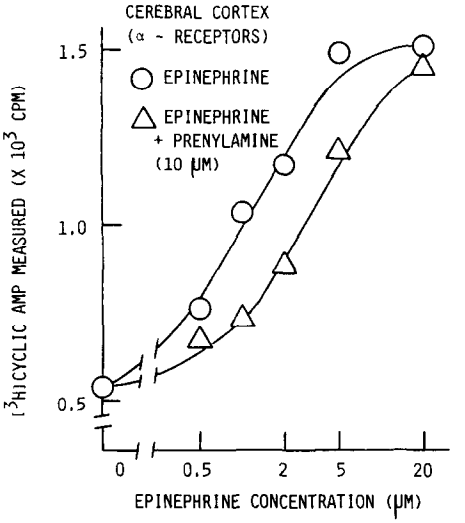

Fig. 4. Competitive inhibition by prenylamine of epinephrine-stimulated cAMP formation via alpha-adrenergic receptors. General conditions were as described in the legend of Fig. 2. Half-maximal activation by epinephrine occurred at 1.2 μM.

Effect of Ca^{2+} *entry blockers on epinephrine-stimulated* [^{3}H]*cAMP levels in vesicles containing beta-adrenergic receptors.* The average of control levels of [^{3}H]cAMP are shown in Table 3. Only prenylamine and, to a lesser extent, nifedipine were inhibitory (Table 5). Prenylamine inhibition was

Table 5. Inhibitory actions of Ca^{2+} entry blockers on epinephrine-stimulated cAMP synthesis via the beta-adrenergic receptor in vesicles from guinea pig cerebellum

Ca^{2+} entry blocker	Change (%) in cAMP by Ca^{2+} entry blockers at concentrations (μM): 3	10	Approx. IC_{50} (μM)
Nifedipine	−1 ± 2	−41 ± 5*	13.
Prenylamine	−27 ± 3†	−74 ± 5*	5.4

Vesicles were prepared and incubated in the presence of Ca^{2+}. Epinephrine was used at 2 μM to stimulate cAMP synthesis. Mean values for all controls used in these experiments are given in Table 3. Calculations were as given in Table 2. Nisoldipine, nitrendipine, diltiazem, verapamil and perhexilene were inactive up to 10, 10, 100, 30 and 10 μM respectively.

* $P < 0.001$.

† $P < 0.01$.

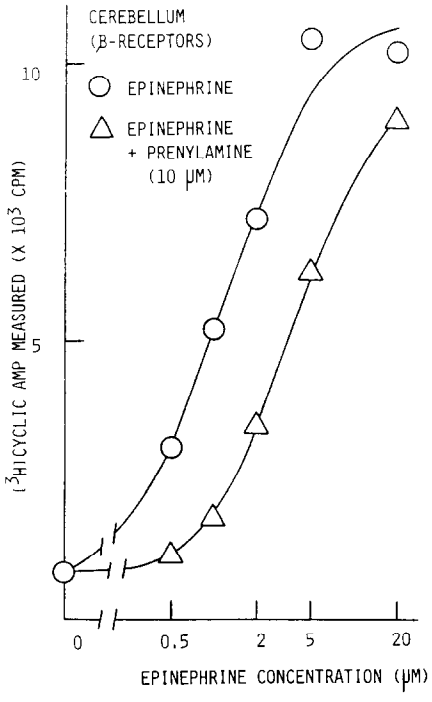

Fig. 5. Competitive inhibition by prenylamine of epinephrine-stimulated cAMP formation via beta-adrenergic receptors. General conditions were as described in the legend of Fig. 2, except that vesicles were prepared from guinea pig cerebellum. Half-maximal activation by epinephrine occurred at 1.1 μM.

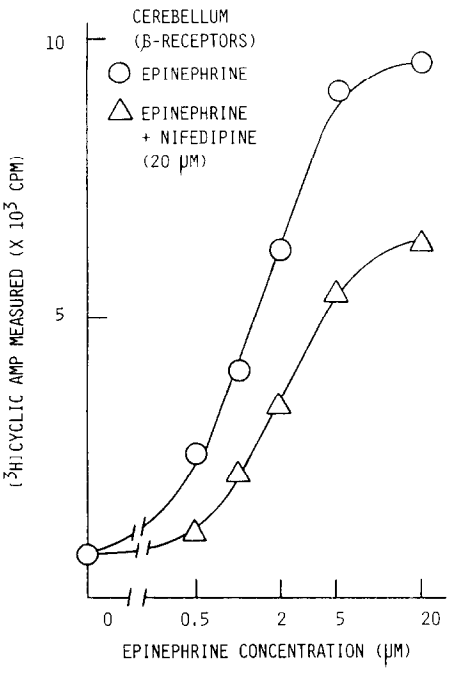

Fig. 6. Non-competitive inhibition by nifedipine of epinephrine-stimulated cAMP formation via beta-adrenergic receptors. General conditions were as described in the legend of Fig. 2, except that vesicles were prepared from guinea pig cerebellum. Half-maximal activation by epinephrine occurred at 1.3 μM.

competitive with epinephrine (Fig. 5), whereas nifedipine inhibition was not competitive (Fig. 6). Although perhexiline has been found to compete with the antagonist [^{3}H]dihydroalprenolol for binding sites believed to be beta-adrenergic receptors [4], it did not block the action of epinephrine in the vesicle beta-adrenergic system. Nisoldipine, nitrendipine, diltiazem and verapamil were also inactive.

DISCUSSION

Antagonism of adenosine by vascular-selective group A Ca^{2+} entry blockers. Only members of this group inhibited the action of adenosine but there was a clear division between drugs such as nifedipine and nisoldipine that were active with approximate IC_{50} values of 5.2 and 7.2 μM, respectively, and nitrendipine which was completely inactive at 10 μM. Structure–activity studies with other analogs (data not shown) indicate that the position of the substituent on the phenyl ring of nitrendipine resulted in inactivity. On the basis of structure we would predict that nimodipine would also be inactive as an adenosine antagonist.

Our findings parallel those of a receptor binding study in which nifedipine and nisoldipine antagonized binding of A_1- and A_2-adenosine receptor radioligands to rat membranes and that other types of Ca^{2+} entry blockers such as verapamil and diltiazem were inactive [8]. However, there were important differences. Nitrendipine was active in the A_2-receptor binding system with an IC_{50} value of 8 μM [8] in contrast to its inactivity at 10 μM in the vesicle system, technically also an A_2-receptor system. Also, although the vesicle system indicated that nifedipine and nisoldipine could antagonize adenosine at equimolar concentrations with the hormone, much higher concentrations of the drugs relative to the concentrations of the radioligands were required for inhibition in the receptor binding system (Table 6). Partly because of this, Murphy and Snyder [8] concluded on the basis of receptor binding experiments tht nifedipine "acted on a site separated from the adenosine receptor but somehow linked to it."

Nevertheless, the action of nifedipine in the vesicle system is consistent with an effect at the receptor. It acted at equimolar concentrations with adenosine and inhibited adenosine competitively so that concentrations of nifedipine that would be required *in*

Table 6. Ratio of IC_{50} values of vascular-selective group A Ca^{2+} entry blockers to adenosine concentration used in vesicle experiments and to adenosine-receptor-radioligand concentrations used in receptor binding experiments

Drug	Adenosine*	[^{3}H]CHA†	[^{3}H]DPX†
Nifedipine	1.0	4,000	250
Nisoldipine	1.4	15,000	1,000
Nitrendipine		60,000	4,000

* Adenosine was used at 5 μM in vesicle experiments. Nitrendipine was inactive at 10 μM, the highest concentration tested.

† Data were calculated from receptor-binding experiments [8]. [^{3}H]CHA ([^{3}H]cyclohexyladenosine) and [^{3}H]-DPX ([^{3}H]1,3-diethyl-8-phenylxanthine) were used at 1 and 2 nM respectively.

vivo for inhibition would be similar to *in vivo* concentrations of adenosine. Also, to the extent studied, nifedipine antagonism of adenosine was independent of an action on Ca^{2+}. Nifedipine similarly inhibited the action of adenosine in vesicles prepared in the presence or absence of Ca^{2+} (Table 2) and thus with markedly different adenylate cyclase activity (Table 1).

Therapeutic significance of adenosine inhibition by nifedipine. Our study provides a biochemical basis for the ability of nifedipine to block the coronary vasodilator action of adenosine *in vivo* [5] since it is effective at concentrations similar to adenosine and acts on both A_1- and A_2-adenosine receptors as defined by receptor binding [8]. Antagonism of endogenous adenosine *in vivo* may explain the superiority of nifedipine to other Ca^{2+} entry blockers in the treatment of patients with atrioventricular conduction disturbances since it did not further block conduction in these patients like other Ca^{2+} entry blockers and as expected by its Ca^{2+} blocking action [13]. Since adenosine slows down conduction [14], nifedipine may have counteracted the action of endogenous adenosine and thus compensated for its own tendency to block conduction by its Ca^{2+}-blocking action. Aminophylline, an adenosine antagonist with adenosine-antagonist potency less than nifedipine, prevents adenosine-induced atrioventricular conduction block [14].

There is evidence that Ca^{2+} entry blockers such as diltiazem, verapamil and nimodipine which are not adenosine antagonists will slow down conduction. Diltiazem and verapamil are useful for treating and preventing atrioventricular nodal reentrant tachycardia [13]. Nimodipine, which is inactive as an A_2-adenosine receptor antagonist and has only slight activity at the A_1-receptor [8], increases the electrocardiographic QT interval in spontaneously beating rabbit heart, suggesting both an increased time necessary for the repolarization of resting membrane and impaired conduction velocity [15].

Recently, the dihydropyridine Ca^{2+} entry blocker Bay K 8644 which has a substituent in the ortho position of the phenyl ring as does nifedipine, and thus a structure consistent with adenosine antagonism, was shown to decrease coronary blood flow in dogs and did not alter intraventricular conduction [16]. Its cardiovascular profile is considered to be a mirror image of that of nifedipine [16]. Nifedipine reduces systemic vascular resistance but has a limited effect on cardiac performance and raised doubts concerning its role as a vasodilating agent in the treatment of chronic congestive heart failure [17]. The adenosine antagonist property of nifedipine and similar structural analogs, as revealed by the vesicle system, may result in a compromised coronary vasodilator action but an absence of decreased atrioventricular conduction characteristic of other Ca^{2+} entry blockers.

Enhancement of [^{3}H]*cAMP levels by group B* Ca^{2+} *entry blockers.* The action of this series, represented by prenylamine and perhexilene, was unique and only occurred in the presence of adenosine (Table 2) and in vesicles formed in the presence of Ca^{2+} (conditions A and B, Tables 1 and 2). Group B drugs were also unique since they were the only Ca^{2+} entry blockers able to selectively inhibit Ca^{2+}- and calmodulin-activated cAMP phosphodiesterase of bovine heart [18]. Our results suggest that this type of phosphodiesterase is active in vesicles during stimulation by adenosine and limits the level of [^{3}H]-cAMP attained. The vesicle system may be a simple method for demonstrating this property of group B Ca^{2+} entry blockers. In turn, these drugs may be useful in revealing the extent to which Ca^{2+}- and calmodulin-activated phosphodiesterase controls the level of adenosine-stimulated cAMP.

Antagonism of epinephrine by nifedipine. Nifedipine blocked the action of epinephrine in the alpha-adrenergic and, to a lesser extent, in the beta-adrenergic vesicle systems (Tables 4 and 5). However, inhibition was not competitive with epinephrine (Figs. 3 and 6) and thus may not have occurred at the adrenergic receptors. This is consistent with the inability of nifedipine to bind to the alpha-adrenergic receptor in rat ventricle [19]. However, the indirect inhibitory action by nifedipine, revealed by the vesicle system, may have pharmacological significance. Abrupt withdrawal of nifedipine results in rebound coronary spasm believed to result partly from an alpha-adrenergic blocking action [20].

Nifedipine may antagonize epinephrine in the adrenergic vesicle systems by two mechanisms. It may block the adenosine receptor, which may mediate the action of the alpha- and, to a lesser extent, the beta-adrenergic receptor on adenylate cyclase [21]. Alternatively, nifedipine may block Ca^{2+} necessary for adrenergic mediated cAMP formation. The need for Ca^{2+} has been shown with brain slices [22]. Nifedipine antagonizes alpha-adrenergic mediated vasoconstriction in rats by preventing the accompanying influx of extracellular Ca^{2+} into vascular smooth muscle [23].

Adrenergic receptor blocking actions of verapamil and prenylamine. Of all the Ca^{2+} entry blockers tested, only verapamil and prenylamine acted as antagonists at adrenergic receptors. Verapamil acted as an alpha-adrenergic receptor antagonist (Table 4) and, because of its affinity for this receptor in receptor binding studies with various tissues [19] and its antagonism of epinephrine in liver cells [2], was not tested further. Prenylamine antagonized both the alpha- and beta-adrenergic vesicle systems (Tables 4 and 5) and was competitive with epinephrine (Figs. 4 and 5), thus consistent with an action at both types of adrenergic receptors. The ineffectiveness of diltiazem as an adrenergic receptor antagonist (Tables 4 and 5) is in agreement with its inability to bind to alpha-adrenergic receptors in human platelets or rat ventricles [19].

Inability of perhexilene to function as a beta-adrenergic receptor antagonist. On the basis of inhibition of [^{3}H]dihydroalprenolol binding, perhexilene acts as a beta-adrenergic receptor antagonist [4]. However, in the present study, perhexilene was ineffective against the normal hormone agonist, epinephrine, in the beta-adrenergic vesicle system (Table 5). Perhexilene was tested at a reasonably high concentration (10 μM). Standard antagonists such as propranolol and labetalol are active at concentrations of 0.0035 and 0.053 μM respectively [11]. Our experience with experimental drugs (data not

shown) indicates that the vesicle system is superior to receptor binding in predicting pharmacological efficacy of putative beta-adrenergic antagonists.

Usefulness of the vesicle system. The vesicle system acts like a miniaturized cell system which can be prepared simply by brief homogenization of brain tissue. The extreme rapidity with which vesicles can be prepared makes it a practical system for studying drugs and may also explain its retention of the biochemical characteristics of intact tissues. Drugs can be tested against normal hormone agonists acting on functional receptors. Vesicles are superior to tissue slices since there are no intercellular spaces with pools of metabolites or neurotransmitters. Vesicles can thus be washed easily and completely, and are immediately accessible to the buffer medium and its additions.

Receptor-binding systems are useful for studying drug interactions with receptors but assumptions have been made that remain unchallenged. For example, the ability of a drug to compete for binding with an antagonist radioligand is considered evidence of the drug's ability to block the normal physiological agonist. Perhexilene competes with [^{3}H]dihydroalprenolol for binding sites believed to be beta-adrenergic receptors in heart and lung membranes [4] but was inactive against epinephrine in the vesicle beta-adrenergic system. The reverse has also been shown. The antidepressant amitryptiline is unable to prevent binding of the H_2-receptor antagonist cimetidine in a guinea pig brain homogenate [24] but is able to block histamine in a gastric mucosal H_2-histamine receptor adenylate cyclase system [25]. It was concluded that cimetidine is a satisfactory ligand for H_2 histamine receptors in guinea pig brain and that antidepressants have only the characteristics of H_1-antagonists [26]. The vesicle system demonstrated both the H_1- and H_2-antagonist properties of amitryptiline and other antidepressants [27].

Although the vesicle system has been useful for the study of other types of drugs, this is its first use for the study of Ca^{2+} entry blockers. It remains to be seen how applicable the data generated from a neuronally-derived system are to drugs presently used as cardiovascular therapeutic agents.

Testing of enantiomers of vascular-selective group A compounds. (+)-Nimodipine is ten times more potent than (−)-nimodipine in inhibiting binding of [^{3}H]nimodipine to red cell ghosts but is five times less potent than (−)-nimodipine in inhibiting binding of [^{3}H]nimodipine to Ca^{2+} channels of skeletal muscle membranes [28]. It would be of interest to test enantiomers of this group of Ca^{2+} entry-blockers on [^{3}H]cAMP levels of adenosine-stimulated vesicles.

Acknowledgements—We thank Elizabeth A. Leszczak and Dr. Jeff B. Meeker for statistical assistance and Rose Prieston for assistance in the preparation of this manuscript.

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