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Anticonvulsant effects of some calcium entry blockers in DBA/2 mice

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1 The behavioural and anticonvulsant effects of several drugs acting by various mechanisms on calcium-channels or affecting intracellular Ca^{2+} concentrations were studied after both systemic and intracerebroventricular administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures.

2 The anticonvulsant effects were evaluated on seizures evoked by means of auditory stimulation (109 dB) in animals placed singly under a perspex dome.

3 Flunarizine and dihydropyridine derivatives, belonging to class I of calcium entry blockers, administered intraperitoneally, were the most potent compounds.

4 Diltiazem, a benzothiazepine derivative belonging to class III, and HA 1004, a calcium antagonist, acting by inhibiting Ca^{2+} mobilization from intracellular stores, injected intraperitoneally, were 3–7.6 fold and 5.8–10.7 fold less potent than flunarizine respectively.

5 Verapamil and methoxyverapamil, two phenylalkylamine derivatives, given intraperitoneally, were completely ineffective in preventing sound-induced seizures in DBA/2 mice. In addition, high doses of verapamil and its methoxyderivative occasionally produced spontaneous tonic-clonic seizures.

6 After intracerebroventricular administration of the hydrosoluble calcium entry blockers, belonging to different classes, the anticonvulsant effects were similar to those observed after systemic administration.

7 The systemic administration of Bay K 8644, a dihydropyridine analogue, having the ability to stimulate calcium entry into cells produced a dose-dependent increase in clonic and tonic convulsions and other neurological side effects.

8 The present results strongly support the idea that some Ca^{2+} antagonists may be useful in human epilepsy.

Introduction

The mechanisms of action of conventional anti-epileptic compounds are complex. Recent *in vitro* studies with phenytoin and carbamazepine have suggested that neuronal calcium-channel blockade may be important in preventing seizure propagation (Sohn & Ferrendelli, 1976; De Lorenzo, 1980; Greenberg *et al.*, 1983). Benzodiazepines also, in rather high concentrations, are able to inhibit the increase in the intracellular Ca^{2+} (De Lorenzo *et al.*,1981). Flunarizine, a diphenylalkylamine derivative which blocks calcium channels, has been shown to possess anticonvulsant properties in several experimental models of epilepsy, i.e. maximal electroshock and pentylenetetrazole-induced seizures, amygdaloid-kindled seizures (Ashton & Wauquier, 1979a,b) and cefazolin-induced seizures in rats (De Sarro, *et al.*, 1986); and myoclonus induced by photic stimulation in the baboon *Papio papio* (De Sarro *et al.*, 1986). It appears to be effective as adjunctive therapy in patients with partial complex seizures (Overweg *et al.*, 1984). In addition, verapamil, nisoldipine and other calcium entry blockers have shown anticonvulsant effects in some animal models i.e., metrazol- or bicuculline-induced seizures and cortical application of penicillin in rats (Kazda *et al.*, 1980; Walden *et al.*, 1984; Wauquier *et al.*, 1985).

Since it is known that calcium antagonists may act

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at different sites of the calcium channel or intracellularly by inhibiting calcium mobilization from intracellular stores (Glossman *et al.*, 1982; Godfraind, 1982a,b; 1984; Van Nueten, 1982) the aim of the present experiments was to assess the antiepileptic activity of some prototypical drugs belonging to the different classes of Ca^{2+} antagonists.

In particular, we studied the effect of flunarizine, nifedipine, nimodipine, nicardipine and nitrendipine as typical drugs belonging to class I, verapamil and methoxyverapamil (D-600) as typical drugs belonging to class II and diltiazem a typical drug of class III according to Fleckenstein (1977). In addition, N-(2'-guanidinoethyl)-5-isoquinolinesulphonamide (HA 1004) which acts by inhibiting Ca^{2+} mobilization from intracellular stores (Asano & Hidaka, 1984; 1985), and Bay K 8644, (1,4 dihydro-2, 6 dimethyl-3-nitro-4-(2-trifluorophenyl) pyridine-5-carboxylic acid), a dihydropyridine analogue, having the ability to stimulate calcium entry into cells (Brown *et al.*, 1984) were used.

The effects of the calcium entry blockers studied, were evaluated in (DBA/2) mice (21–28 days old) genetically susceptible to sound-induced seizures both after their intracerebroventricular (i.c.v.) administration as well as after systemic administration (i.p.). This could permit us to ascertain possible differences between the various classes of calcium antagonists. A preliminary account of some of these experiments has been given to the British Pharmacological Society (Ascioti *et al.*, 1986).

Methods

DBA/2 mice, 21–28 days old, weight 6–12 g, purchased from Charles River (Colco, Como, Italy) were exposed to auditory stimulation (109 dB for 60 s or until tonic extension occurred), 45 min after i.p. administration of saline, vehicle or drugs or 30 min after intracerebroventricular (i.c.v.) injection of phosphate buffer or several hydrosoluble calcium entry blockers belonging to different classes. Intracerebroventricular injections were performed under light ether anaesthesia direct into the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to bregma; depth 2.4 mm) by use of a Hamilton microsyringe (type 701N) fitted with a nylon cuff on the needle.

The hydrosoluble compounds, diltiazem, HA 1004, nicardipine and verapamil, were dissolved in sodium phosphate buffer 0.67 mM, pH 7.4, and animals were injected intraventricularly with 10 µl of vehicle (buffer) or drug solution. For systemic injections, flunarizine, nifedipine, nimodipine, nitrendipine Bay K 8644 and D-600 were given intraperitoneally (0.1 mg 10 g^{-1} of body weight of the mouse) as a freshly ultrasonicated suspension in 90% saline, 5% polyethylene glycol and 5% ethanol. Diltiazem, nicardipine, HA 1004 and verapamil were dissolved in sterile saline. Some of these compounds are sensitive to light so weighing and handling were carried out under light from sodium vapour lamps and the substances were protected from light during the experiments. Individual animals were placed under a perspex dome (diameter 58 cm) and 30 s allowed for habituation and assessment of locomotor activity. Auditory stimulation (109 dB) was applied for 60 s or until tonic extension occurred. Seizure response (SR) as previously reported (De Sarro *et al.*, 1984) was assessed on the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonic, 4 = respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing with an Elektrolaboratoriet thermometer type T.E.3. Behavioural changes were observed during the period between drug administration and auditory testing.

Statistical comparisons between groups of control and drug-treated animals were made with Fisher's exact probability test (incidence of the seizure phases) or ANOVA and Dunnett's *t* test (rectal temperatures). The percentage incidence of each phase of the audiogenic seizure was determined for each dose of calcium antagonist administered and log dose-response curves were fitted by linear regression analysis of probit-transformed percentage response. ED_{50} values (with 95% fiducial limits) for each compound and each phase of seizure response were estimated by the method of probit analysis (Finney, 1978); the relative anticonvulsant activities were determined by comparison of respective ED_{50} values.

The sources of the drugs used were: flunarizine (Polifarma Res. Labs., Rome, Italy), nifedipine (Sigma, St. Louis, MO, U.S.A.), nicardipine (Sandoz Res. Labs., Basel, Switzerland), nimodipine (Bayer Res. Labs, Milan, Italy), nitrendipine (Recordati Res. Labs., Milan, Italy), diltiazem (Sigma-Tau Res. Labs., Pomezia, Italy), verapamil (Knoll Pharmaceutical Company, Ludwigshafen, West Germany), D-600 (methoxy-verapamil, Knoll Pharmaceutical Company, Ludwigshafen, West Germany), HA 1004 (N-(2-guanidinoethyl-5- isoquinolinesulphonamide), Asahi Chemical Industry, Tokyo, Japan) and Bay K 8644 (1,4-dihydro-2, 6-dimethyl-3- nitro-4-(2-trifluorophenyl) pyridine-5-carboxylic acid, Bayer Res. Labs., Milan, Italy).

Results

The influence of the calcium entry blockers on the audiogenic seizure response varied according to the different classes.

Intraperitoneal administration

Diphenylalkylamine: Flunarizine (10.5–84 μmol kg^{-1}, i.p.), produced significant protection ($P<0.05$) against the tonic phase of audiogenic seizure response in DBA/2 mice 45 min after administration (Table 1). Doses of 21–84 μmol kg^{-1} significantly reduced the incidence of clonic phase, whereas a significant protection against the wild running phase was seen after flunarizine 42 and 84 μmol kg^{-1} only (Table 1). Flunarizine (42 and 84 μmol kg^{-1}, i.p.) reduced the locomotor activity in all animals, sedation was observed in a few animals (2/10 and 4/10 after 42 and 84 μmol kg^{-1} respectively). Ataxia and a significant fall in rectal temperature were also evident following the highest dose (84 μmol kg^{-1}, i.p.) of flunarizine. Probit analysis of the data indicated the following ED_{50} values (± 95% fiducial limits) for

Table 1 The effect of flunarizine, nifedipine, nicardipine, nimodipine and nitrendipine on audiogenic seizures in DBA/2 mice

Drug	*Dose* (μmol kg^{-1})	*% response* *WR*	*Clonus*	*Tonus*	*RA*	*SR*	*Temp* (°C) Mean ± s.e.mean	n
Flunarizine	Vehicle	100	100	80	40	3.2	37.5 ± 0.08	10
	2.1	100	100	80	60	3.4	37.7 ± 0.18	10
	5.25	100	90	60	60	3.1	37.1 ± 0.22	10
	10.5	90	70	20**	10	1.9	37.7 ± 0.28	10
	21	70	40**	0**	0	1.1	37.2 ± 0.25	10
	42	40**	20**	10**	10	0.8	37.1 ± 0.25	10
	84	20**	10**	0	0	0.3	36.2 ± 0.3†	10
Nifedipine	Vehicle	100	100	90	50	3.4	38.9 ± 0.15	10
	10.5	100	90	80	40	3.1	38.3 ± 0.18	10
	21	100	80	60	40	2.8	38.3 ± 0.19†	10
	42	90	50*	30**	20	1.9	38.1 ± 0.16†z	10
	84	50*	10**	0**	0**	0.6	37.2 ± 0.18†z	10
	126	20**	0**	0**	0**	0.2	36.9 ± 0.1†z	10
Nicardipine	Vehicle	100	100	100	50	3.5	38.6 ± 0.17	10
	10.5	100	90	80	50	3.2	37.5 ± 0.28	10
	21	100	60	50*	50	2.6	37.2 ± 0.31†	10
	42	90	50*	40**	40	2.3	36.9 ± 0.33†	10
	84	60	40**	0**	0**	1.0	36.8 ± 0.26†	10
	126	40**	10**	0**	0**	0.5	36.6 ± 0.24†z	10
	168	0**	0**	0**	0**	0	36.2 ± 0.26†z	10
Nimodipine	Vehicle	100	100	100	40	3.4	38.3 ± 0.21	10
	21	100	80	70	30	2.8	38.2 ± 0.13	10
	42	60	50*	20**	20	1.5	37.7 ± 0.18	10
	48	40**	20**	0**	0	0.6	36.9 ± 0.33††	10
	126	10**	0**	0**	0	0.1	36.3 ± 0.3††	10
Nitrendipine	Vehicle	100	100	100	60	3.6	38.4 ± 0.14	10
	21	100	90	70	40	3.0	38.2 ± 0.37	10
	42	100	80	50*	50	2.8	37.73 ± 0.16	10
	84	90	50*	30**	20	1.9	37.22 ± 0.12††	10
	126	80	30**	0**	0**	1.1	37.3 ± 0.18††	10
	168	40**	10**	0**	0**	0.5	36.9 ± 0.15††	10

Groups of DBA/2 mice were injected i.p. with the stated doses of the drugs or vehicle (control) and exposed to auditory stimulation 45 min after drug injection. Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by $*P<0.05$; $**P<0.01$.

WR = wild running; RA = respiratory arrest; arithmetic mean of the maximum individual responses for each animal in the group. Temp is the rectal temperature (mean ± s.e.mean) measured immediately before auditory stimulation. Significant differences between rectal temperature in drug-treated and control group are denoted by $\dagger P<0.05$; $\dagger\dagger P<0.01$.

flunarizine against the main phases of the audiogenic seizure response: tonic 7 (5–10) μmol kg^{-1}; clonic 21 (14–32) μmol kg^{-1}; wild running 37 (27–54) μmol kg^{-1}.

Dihydropyridines: Significant reduction of the incidence ($P<0.05$) of the tonic and clonic phases was observed 45 min after nifedipine (42–126 μmol kg^{-1}, i.p.) whereas significant protection against the initial wild running phase was observed after nifedipine 84 and 126 μmol kg^{-1} i.p. only. Lower doses (10.5 and 21 μmol kg^{-1}, i.p.) had no effect on the behaviour of mice and did not protect against audiogenic seizures (Table 1). A significant fall in rectal temperature was evident following nifedipine 84 and 126 μmol kg^{-1}, i.p. Behavioural changes after the latter dose included piloerection and ataxia (70% of animals).

Probit analysis of the data indicated the following ED_{50} values (± 95% confidence limits) for nifedipine against the principal phase of the audiogenic seizure response: tonic 21 (12–36) μmol kg^{-1}; wild running 94 (64–138) μmol kg^{-1}.

Following systemic administration, nicardipine (42–168 μmol kg^{-1}, i.p.) suppressed all phases of the audiogenic seizure response in a dose-dependent manner (Table 1). In particular, significant reduction ($P<0.05$) of clonic and tonic phases of audiogenic seizure occurred after the intraperitoneal administration of nicardipine (42 and 84 μmol kg^{-1}). Following the highest doses used (126 and 168 μmol kg^{-1}, i.p.) the incidence of all phases of the seizure response was significantly ($P<0.01$) suppressed in the absence of behavioural or postural side-effects (Table 1). The mean rectal temperatures of nicardipine-treated mice were significantly lowered ($P<0.05$) after 21–168 μmol kg^{-1}, i.p.

ED_{50} values (± 95% confidence limits) for nicardipine against the sound-induced seizures in DBA/2 mice were as follows: tonic 21 (15–32) μmol kg^{-1}; clonic 33 (19–57) μmol kg^{-1}; wild running 96 (73–126) μmol kg^{-1}.

Nimodipine (42–126 μmol kg^{-1}, i.p.) produced significant protection ($P<0.05$) against the tonic and clonic phases of audiogenic seizure response in DBA/2 mice 45 min after administration (Table 1). Doses of 84 and 126 μmol kg^{-1} i.p. significantly reduced ($P<0.01$) the incidence of all phases of the seizure response. These latter doses induced a significant fall in rectal temperature. Reduction of locomotor activity, mild ataxia and sedation was also evident in 8 out of 10 mice and in all mice following nimodipine 84 and 126 μmol kg^{-1} i.p. respectively.

ED_{50} values (± 95% fiducial limits) for nimodipine against the principal phases of the audiogenic seizure response were as follows: tonic 29 (18–47) μmol kg^{-1}; clonic 51 (37–71) μmol kg^{-1}; wild running 71 (49–103) μmol kg^{-1}.

After nitrendipine (84 and 126 μmol kg^{-1}, i.p.) tonic and clonic phases of the seizure response were significantly ($P<0.05$) depressed (Table 1). Nitrendipine (168 μmol kg^{-1}, i.p.) produced significant protection against all phases of the audiogenic seizure ($P<0.01$). However, lower doses (21 and 42 μmol kg^{-1}, i.p.) did not protect mice against audiogenic seizures (Table 1).

In addition, following 84, 126 or 168 μmol kg^{-1}, i.p. of nitrendipine many animals showed piloerection, a significant fall in rectal temperature, ataxia and some reduction in locomotor activity.

Probit analysis of the data indicated the following ED_{50} values (± 95% confidence limits) for nitrendipine against the principal phases of the audiogenic seizure response: tonic 46 (27–78) μmol kg^{-1}; clonic 84 (53–133) μmol kg^{-1}; wild running 147 (117–184) μmol kg^{-1}.

Dibenzothiazepine Following diltiazem (84 μmol kg^{-1}, i.p.) both the clonic and tonic phases of the seizure response were suppressed in the absence of the behavioural or postural side effects (Table 2). Significant reduction ($P<0.05$) of all phases of the seizure response occurred following diltiazem 126 and 168 μmol kg^{-1}, i.p. However, after lower doses (21 and 42 μmol kg^{-1}, i.p.) no significant anticonvulsant activity was observed. The mean rectal temperatures of diltiazem-treated mice were significantly lowered ($P<0.05$) after 84–168 μmol kg^{-1}, i.p.

Probit analysis of the data indicated the following ED_{50s} values (± 95% fiducial limits) for diltiazem against the sound-induced seizures in DBA/2 mice: tonic 53 (37–76) μmol kg^{-1}; clonic 63 (35–113) μmol kg^{-1}; wild running 115 (85–156) μmol kg^{-1}.

Phenylalkylamines: No significant anticonvulsant effect was shown after verapamil (42–126 μmol kg^{-1}, i.p.) (Table 2). A dose-dependent convulsant activity was observed shortly after administration of verapamil 105 and 126 μmol kg^{-1} with mice showing a period of hyperactivity followed by clonic activity of the hind limbs, generalized myoclonus, loss of righting reflex, circling activity, barrel rolling and tonic flexion and extension of limbs. Respiratory arrest and death occurred before the application of auditory stimulus in 8/10 and 10/10 members of the groups treated with 105 and 126 μmol kg^{-1} respectively.

In addition, no anticonvulsant properties were seen after D-600 (2.1–8.4 μmol kg^{-1}, i.p.) (Table 2). However, a dose-dependent convulsant activity, similar to that observed after the highest doses (105 and 126 μmol kg^{-1}) of verapamil was evident within 5–10 min after the injection of 21 and 42 μmol kg^{-1} i.p. of D-600. Respiratory arrest and death occurred in all mice treated with the highest doses (21 and 42 μmol kg^{-1} i.p.) of D-600 before the auditory test. The mean rectal temperatures were significantly

lowered ($P<0.05$) after verapamil (84 μmol kg^{-1}, i.p.) and D-600 (2.1, 4.2 and 8.4 μmol kg^{-1}, i.p.).

HA 1004 (42 and 84 μmol kg^{-1}, i.p.) did not protect mice against all phases of audiogenic seizures. However, after 126 and 168 μmol kg^{-1}, i.p., tonic and clonic phases of the seizure response were significantly depressed ($P<0.05$) although no significant reduction in the incidence of wild running phase was observed at dose levels studied (Table 2). No ataxia or other side effects were evident after HA 1004 administration. A significant fall ($P<0.05$) in rectal temperature was observed after HA 1004 168 μmol kg^{-1}, i.p., only.

ED_{50} values (± fiducial limits) for HA 1004 against the principal phases of the audiogenic seizure response in DBA/2 mice were as follows: tonic 75 (48–117) μmol kg^{-1}; clonic 122 (91–165) μmol kg^{-1}.

Bay K 8644 (10.5–84 μmol kg^{-1}, i.p.) did not significantly modify the occurrence of wild running clonic and tonic phases of audiogenic seizures in DBA/2 mice. However a dose-dependent increase in the incidence of respiratory arrest was evident.

Table 2 The effect of diltiazem, verapamil methoxyverapamil, (D-600), HA 1004 and Bay K 8644 on audiogenic seizures in DBA/2 mice

Drug	*Dose* (μmol kg^{-1})	*% response* *WR*	*Clonus*	*Tonus*	*RA*	*SR*	*Temp* (°C) Mean ± s.e.mean	n
Diltiazem	Saline	100	100	100	50	3.5	38.6 ± 0.18	10
	21	100	90	90	50	3.3	38.3 ± 0.21	10
	42	100	70	70	50	2.9	37.3 ± 0.29	10
	84	90	30**	10**	0**	1.3	36.6 ± 0.21††	10
	126	50*	10**	0**	0**	0.6	36.6 ± 0.14†	10
	168	0**	0**	0**	0**	0	35.8 ± 0.42††	10
Verapamil	Saline	100	100	80	40	3.2	38.8 ± 0.28	10
	42	100	80	80	80	3.4	38.2 ± 0.22	10
	63	80	80	80	80	3.2	38.1 ± 0.25	10
	84	70	60	60	50	2.4	37.7 ± 0.2††	10
	105	8/10 died before test						
	126	10/10 died before test						
D-600	Vehicle	100	100	90	50	3.4	38.7 ± 0.18	10
	2.1	100	100	80	40	3.2	38.1 ± 0.10†	10
	4.2	90	70	50	20	2.3	38.0 ± 0.08††	10
	8.4	70	50	40	20	1.8	37.7 ± 0.16††	10
	21	8/8 died before test						8
	42	8/8 died before test						8
HA 1004	Saline	100	100	80	50	3.3	37.9 ± 0.1	10
	42	100	100	70	50	3.2	38.0 ± 0.17	10
	84	100	80	50	30	2.6	37.8 ± 0.11	10
	126	100	50**	40*	20	2.2	37.6 ± 0.16	10
	168	100	20**	10**	10	1.4	37.5 ± 0.07†	10
BAY K 8644	Vehicle	100	100	100	30	3.3	38.02 ± 0.16	10
	10.5	100	100	80	10	2.9	37.6 ± 0.14	10
	21	100	100	100	30	3.3	37.5 ± 0.25	10
	42	100	100	100	50	3.5	37.6 ± 0.17	10
	84	100	100	100	70	3.7	37.0 ± 0.13††	10

Groups of DBA/2 mice were injected i.p. with the stated doses of the drugs or saline (or vehicle) and exposed to auditory stimulation 45 min after drug injection. Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control and drug-treated group are denoted by $*P<0.05$; $**P<0.01$.

WR = wild running; RA = respiratory arrest; SR = the arithmetic mean of the maximum individual responses for each animal in the group. Temp is the rectal temperature (mean ± s.e.mean) measured immediately before auditory stimulation. Significant differences between rectal temperature in drug-treated and control group are denoted by $\dagger P<0.05$; $\dagger\dagger P<0.01$.

Bay K 8644 (10.5–84 μmol kg^{-1}, i.p.) produced marked behavioural effects in DBA/2 mice 10–17 min after injection. In particular, a decrease of locomotor activity, in comparison to vehicle-treated animals, was observed after the lower dose (10.5 μmol kg^{-1}) of Bay K 8644. Mice treated with 21, 42 and 84 μmol kg^{-1}, i.p., showed a dose-dependent ataxia with splayed hind limbs and delay of appearance of the righting reflex (which was never completely lost.)

In addition, after the latency period the mice showed arching back, tremor, Straub tail, ptosis, vocalization, jumping and clonus of fore limbs. These phenomena were dose-dependent and lasted 40–55 min. Tonic convulsions were observed in 2 out of 10 and 4 out of 10 mice treated with 42 and 84 μmol of Bay K 8644 respectively. The mean rectal temperatures were significantly lowered ($P<0.01$) after Bay K 8644 μmol kg^{-1}, i.p., only.

Intracerebroventricular administration

The anticonvulsant activity of some classes of calcium antagonists was also evaluated after intracerebral administration of a hydrosoluble compound for each class of calcium entry blockers administered intraperitoneally.

Dihydropyridines: Nicardipine (0.17–10.5 μmol, i.c.v.) induced a dose-dependent reduction of all phases of audiogenic seizure. A significant decrease ($P<0.05$) of clonic and tonic phase was observed with nicardipine (0.42–10.5 μmol, i.c.v.), although significant protection against the wild running phase was evident after the higher dose of nicardipine (10.5 μmol i.c.v.) only. No significant changes in body temperature, ataxia or other neurological side effects were evident after i.c.v. administration of nicardipine (Table 3). ED_{50} values (± fiducial limits) for nicardipine i.c.v. against the principal phases of audiogenic seizures in DBA/2 mice were as follows: tonic 0.45 (0.29–0.7) μmol; clonic 0.49 (0.3–0.79) μmol; wild running 7.22 (5.1–10.2) μmol.

Dibenzothiazepines: Following diltiazem (4.2–12.6 μmol, i.c.v.) no significant reduction in the incidence of wild running, clonic and tonic phase was recorded, whereas at the higher dose of diltiazem (16.8 μmol) 7/10 and 5/10 mice did not show, in comparison to control group, the tonic and clonic phase respectively (Table 3). No significant fall in rectal temperature or neurological side effects were observed after i.c.v. administration of this compound.

The ED_{50} value (± 95% confidence limits) for diltiazem i.c.v. against the tonic phase of audiogenic seizures in DBA/2 mice was 12.4 (8.9–17.3) μmol.

Phenylalkylamines: Verapamil (0.05–0.5 μmol, i.c.v.) did not significantly protect mice against audiogenic seizures. A significant reduction ($P<0.01$) in the incidence of clonic and tonic phase of seizure response was seen after verapamil 1 μmol i.c.v. only. However, a dose-dependent convulsant activity, similar to that observed after the intraperitoneal administration of the highest doses of verapamil (105 and 126 μmol kg^{-1}) was observed 12–15 min after i.c.v. administration of verapamil. In addition, respiratory arrest and death occurred in 2/10 and 5/10 members of the groups treated with 0.5 and 1.0 μmol respectively. A significant fall ($P<0.05$) in rectal temperature was also evident after the two higher doses of verapamil (0.5 and 1.0 μmol, i.c.v.).

HA 1004: A significant reduction ($P<0.05$) in the incidence of clonic and tonic phase was noted after i.c.v. administration of HA 1004 (12.6 μmol) (Table 3). Significant reduction of all phases of the seizure response occurred after the highest dose studied (16.8 μmol, i.c.v.). However, lower doses (1.68, 4.2 and 8.4 μmol, i.c.v.) did not protect mice against audiogenic seizures (Table 3). The mean rectal temperatures were significantly lowered ($P<0.01$) after HA 1004 (8.4, 12.6 and 16.8 μmol, i.c.v.).

Probit analysis of the data indicated the following ED_{50} values (± 95% confidence limits) for i.c.v. administration of HA 1004 against the audiogenic seizure phases: tonic 7.4 (5.3–11.3) μmol; clonic 7.6 (5.2–11.4) μmol; wild running 15.5 (9.7–24.7) μmol.

Discussion

The present results show that some calcium antagonists given systemically or into the lateral cerebral ventricle possess anticonvulsant effects in a susceptible strain (DBA/2) of mice, thus confirming previous data showing anticonvulsant activity in other models of experimental epilepsy, i.e. cefazolin-induced seizures in rats (Rotiroti *et al.*, 1983; De Sarro *et al.*, 1986), amygdaloid-kindled rats (Ashton & Wauquier, 1979b), amygdaloid-kindled dogs (Wauquier *et al.*, 1979), myoclonus induced by photic stimulation in the baboon *Papio papio* (De Sarro *et al.*, 1986), allylglycine-induced seizures in rats (Ashton & Wauquier, 1979a) and bicuculline- or metrazol-induced seizures in rats (Wauquier *et al.*, 1985).

In addition, the dose-response curves for various classes of calcium antagonists after their systemic administration reveal that flunarizine, a calcium antagonist having a diphenylalkylamine structure, is significantly more active than the dihydropyridine derivatives, nifedipine, nimodipine, nicardipine and nitrendipine, although it is believed that both groups share a common mechanism (Class I) showing a specific binding or action at the outer mouth of the

Table 3 Influence of diltiazem, HA 1004, nicardipine and verapamil on the development of audiogenic seizures in DBA/2 mice

Drug	*Dose* (μmol kg^{-1})	*WR*	*% response* *Clonus*	*Tonus*	*RA*	*SR*	*Temp* (°C) Mean ± s.e.mean	n
Phosphate buffer		100	100	100	50	3.5	37.9 ± 0.21	10
Diltiazem	4.2	100	100	100	30	3.3	37.8 ± 0.22	10
	8.4	100	80	60	30	2.7	38.8 ± 0.19	10
	12.6	100	60	50*	20	2.3	37.0 ± 0.23	10
	16.8	100	50*	30**	20	2.1	38.1 ± 0.21	10
Phosphate buffer		100	100	90	60	3.5	37.9 ± 0.16	10
HA 1004	1.68	100	100	80	30	3.6	37.8 ± 0.10	10
	4.2	100	80	80	20	2.8	37.8 ± 0.15	10
	8.4	90	70	60	20	2.5	37.2 ± 0.13†z	10
	12.6	70	40**	30**	0**	1.4	37.0 ± 0.15†z	10
	16.8	40**	0**	0**	0**	0.4	36.7 ± 0.19†z	10
Phosphate buffer		100	100	100	60	3.6	37.9 ± 0.16	10
Nicardipine	0.17	100	80	70	40	2.9	38.0 ± 0.19	10
	0.42	100	50*	50*	20	2.2	38.2 ± 0.18	10
	1.05	100	40**	30**	10	1.8	38.1 ± 0.17	10
	2.1	100	20**	10**	0**	1.3	38.0 ± 0.13	10
	5.3	70	10**	0**	0**	0.8	37.8 ± 0.24	10
	10.5	40**	0**	0**	0**	0.4	37.6 ± 0.21	10
Phosphate buffer		100	100	100	30	3.3	37.8 ± 0.19	10
Verapamil	0.05	100	80	80	40	3.0	37.7 ± 0.21	10
	0.1	90	70	60*	20	2.4	37.4 ± 0.13	10
	0.5§	75	63	63*	50	3.13	36.8 ± 0.32†	8
	1.0§	60	40**	40**	20	3.1	36.4 ± 0.18†z	5

Groups of 10 mice were injected i.c.v. with diltiazem, HA 1004, nicardipine or verapamil and auditory stimulation was commenced 30 min later. The table shows the percentage incidence of the principal phases of the audiogenic seizure response in groups of control and drug-treated animals.
*$P<0.05$; **$P<0.01$. WR = wild running; RA = respiratory arrest; SR = mean maximal seizure response (see Methods for grading). Significant differences between rectal temperature in drug-treated and control group are denoted by †$P<0.05$; ††$P<0.01$. §Respiratory arrest and death occurred before the auditory test in 2/10 and 5/10 mice treated with verapamil 0.5 and 1.0 μmol respectively.

Ca^{2+} channel (Fleckenstein, 1977; Triggle, 1981; Glossman *et al.*, 1982). In fact, Class I drugs compete with [^{3}H]-nimodipine binding in guinea-pig brain membranes in a monophasic manner (Hill coefficients ~1.0) (Ferry & Glossmann, 1982). In addition, the log dose-response curves for flunarizine and the dihydropyridines do not differ significantly from parallelism and this is consistent with the concept that the anticonvulsant activity of these agents is mediated by a common mechanism.

In comparison to the previous groups of calcium antagonists, diltiazem, a benzothiazepine derivative belonging to Class III (Fleckenstein, 1977) and believed to act at the inner mouth of the Ca^{2+} channel is less effective. Comparing the ED_{50} values after intraperitoneal administration, diltiazem is 3.0–7.6 times less potent than flunarizine and 1.2–1.8 times less than nifedipine, although the dose-response curve of the anticonvulsant effect of diltiazem almost overlaps with that of nitrendipine.

Conversely phenylalkylamine derivatives (verapamil and its methoxy-derivative D-600) belonging to Class II and believed to act similarly to diltiazem at the inner mouth of the Ca^{2+} channel (Glossman *et al.*, 1982; Godfraind, 1982a,b; 1984) are ineffective in preventing sound-induced seizures in DBA/2 mice.

HA 1004, a calcium antagonist, acting by inhibiting Ca^{2+} mobilization from intracellular stores and described as a selective calmodulin antagonist (Asano & Hidaka, 1984; 1985) given intraperitoneally is active as an anticonvulsant for the clonic and tonic phases only, although its potency is 5.8–10.7 times lower than that

of flunarizine and similar (half as potent) to that of diltiazem and nitrendipine.

Intraventricular administration of nicardipine, a dihydropyridine derivative (0.42–10.5 μmol) and HA 1004 (12.6 and 16.8 μmol) prevents all the phases of audiogenic seizures, whereas diltiazem and verapamil, given at similar or higher doses than nicardipine and HA 1004 are ineffective against the wild running phase of the audiogenic seizures.

Clearly, different classes of Ca^{2+} antagonists acting at the Ca^{2+} channel, or intracellularly, are effective as anticonvulsants, indicating that inhibition of Ca^{2+} influx or prevention of Ca^{2+} release from intracellular stores can antagonize the enhanced excitatory transmission occurring in DBA/2 mice after auditory stimulation. The anticonvulsant potency of valproate and its analogues seems to be related to their ability to reduce brain aspartate level and to a lesser extent with their ability to elevate the γ-aminobutyric acid (GABA) level (Chapman *et al.*, 1983). Although there have been no investigations into possible abnormalities in the aspartatergic and glutamatergic transmitter systems in DBA/2 mice, it is likely that in these sound-sensitive mice excitatory mechanisms culminating in convulsions may be due to Ca^{2+}-dependent release of excitatory transmitters i.e. glutamate or aspartate, thus explaining the efficacy of calcium antagonists in this model. This concept is supported by the finding that synaptic release of excitatory amino acids may contribute to initiation and to spread of seizures (Meldrum, 1986). That such mechanisms contribute to reflex epilepsy is also indicated by the anticonvulsant action of antagonists of excitatory amino acids in DBA/2 mice (Croucher *et al.*, 1982; Chapman *et al.*, 1984a; Croucher *et al.*, 1984a,b; Jones *et al.*, 1984).

The reason for the differences in anticonvulsant activity obtained with the various classes of Ca^{+} antagonists is not known so far, although it seems clear that Ca^{2+} antagonists acting at the outer site of the calcium channel (flunarizine and dihydropyridines) are more powerful than drugs acting at the inner mouth (diltiazem and verapamil) or intracellularly (HA 1004). In addition, flunarizine appears in our study to be significantly more powerful than the dihydropyridines, thus suggesting that it may possess different pharmacokinetic and lipophylic properties which make the compound more accessible to the brain-stem and mid-brain structures involved in the pathophysiology of sound evoked seizures (Meldrum *et al.*, 1983b; Croucher *et al.*, 1983). Alternatively, a possible explanation for the more powerful anticonvulsant action of flunarizine is that this compound exerts its effects primarily under conditions where calcium and/or sodium influx is pathologically increased as after ischaemia or pharmacological stimulation or during seizure activity (Holmes *et al.*, 1984; Wauquier *et al.*, 1985). The lack of action of verapamil after peripheral administration appears not to be due to its poor penetration into the brain (Doran *et al.*, 1985); as it is also ineffective after i.c.v. administration. The toxic effects of D-600, following i.p. administration, are consistent with a previous study (Wauquier *et al.*, 1985). As far as diltiazem is concerned its lack of protection against the wild running phase of audiogenic seizures after i.c.v. administration in comparison to its weak activity after i.p. injection, may indicate that this compound does not affect the brain areas involved in the pathophysiology of sound-induced seizures after i.c.v. injection. Alternatively, the anticonvulsant activity of diltiazem could be due to a metabolite formed peripherally. The lower anticonvulsant activity of HA 1004, an intracellular calcium antagonist acting as a selective calmodulin antagonist, suggests that an involvement of the Ca^{2+}-calmodulin system is not primarily important for the anticonvulsant effects of some calcium antagonists in audiogenic seizures of DBA/2 mice. This finding is quite interesting since concentrations of diphenylhydantoin, carbamazepine and benzodiazepines that are anticonvulsant against electroshock seizures, potently inhibit the phosphorylation of some proteins regulated by a Ca^{2+}-calmodulin system (De Lorenzo, 1980; De Lorenzo *et al.*, 1981).

A role for benzodiazepines as regulators of Ca^{2+} channel activity has been suggested. Diazepam competitively inhibits [^{3}H]-nitrendipine binding (Ferrendelli & Daniels-McQueen, 1982; Taft & De Lorenzo, 1984; Rampe & Triggle, 1986). In addition, the effects of micromolar concentrations of diazepam on synaptosomal ^{45}Ca uptake are reversed by the 1,4-dihydropyridine calcium channel antagonists, nifedipine and nitrendipine (Mendelson *et al.*, 1984). Micromolar concentrations of benzodiazepines are necessary to inhibit Ca^{2+} conductance in neurones and maximum electroshock-induced seizures, kindling seizures in animals (Johansen *et al.*, 1985) and to prevent the generalized seizures of human epilepsy (see Rampe & Triggle, 1986).

In conclusion, the present experiments show that Ca^{2+} antagonists given systemically or i.c.v. to DBA/2 mice possess anticonvulsant effects in doses, comparable with those reported to be active in other experimental models of epilepsy. The dose levels of calcium antagonist are quite similar to those used for other antiepileptic drugs (Chapman *et al.*, 1984a) such as sodium valproate (Chapman *et al.*, 1984b) excitatory amino acid antagonists (Croucher *et al.*, 1982; Meldrum *et al.*, 1983a,b) and drugs enhancing GABAergic transmission (Horton *et al.*, 1979 Croucher *et al.*, 1983). The reduction of locomotor activity and ataxia, usually observed with other anticonvulsant drugs, are less marked after adminis-

tration of Ca^{2+} antagonists. Our results suggest that the anticonvulsant activity of Ca^{2+} antagonists against the audiogenic seizure phases is temperature-independent. In fact, compounds which induced a significant fall in rectal temperature (verapamil, D-600 and Bay K 8644) showed no significant anticonvulsant activity. The site of anticonvulsant action of calcium entry blockers remains to be elucidated. The present finding that Ca^{2+} activation by Bay K 8644 induces in DBA/2 mice wild running, rearing, jumping, ataxia, Straub tail and tonic-clonic convulsions which are antagonized by nifedipine (unpublished data) is consistent with a previous study (Bolger *et al.*, 1985). These data indicate that Ca^{2+} plays an important role in the pathophysiology of epileptic disorders and suggest that some Ca^{2+} antagonists may be useful in the therapy of some types of human epilepsy.

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