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The effects of ifenprodil and eliprodil on voltage-dependent Ca^{2+} channels and in gerbil global cerebral ischaemia

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Abstract

Ifenprodil and eliprodil are both non-competitive NMDA receptor antagonists which have been shown to inhibit neuronal Ca^{2+} channel currents. We have examined the effects of these agents on two defined subtypes of voltage-dependent Ca^{2+} channels and in the gerbil model of global cerebral ischaemia. Recombinantly expressed human $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$ Ca^{2+} subunits in HEK293 cells, which results in an ω-conotoxin-sensitive neuronal N-type voltage-dependent Ca^{2+} channel and ω-Aga IVA sensitive Ca^{2+} channels (P-type) in acutely isolated cerebellar Purkinje neurones were reversibly inhibited by ifenprodil and eliprodil. Human N-type Ca^{2+} channel currents were inhibited by ifenprodil and eliprodil with IC_{50} values of 50 μM and 10 μM respectively whereas P-type Ca^{2+} channel currents were inhibited reversibly by ifenprodil and eliprodil with approximate IC_{50} values of 60 μM and 9 μM respectively. Maximum current block observed for both channel subtypes was approximately 80% for both ifenprodil and eliprodil. For neuroprotection studies, animals were subjected to 5 min bilateral carotid artery occlusion with or without administration of either ifenprodil or eliprodil (5, 10 or 20 mg/kg i.p.) immediately after surgery followed by two further doses (2.5, 5 or 10 mg/kg, respectively) at 3 and 6 h post-occlusion. Both compounds provided significant protective effects against ischaemia-induced neurodegeneration in the CA1 region of the hippocampus. These results indicate that both ifenprodil and eliprodil protect against ischaemia-induced neurodegeneration when administered post-occlusion and that they also block N and P-type voltage-dependent Ca^{2+} channels.

Keywords: HEK293 cell; Ca^{2+} channel, voltage-dependent; NMDA receptor antagonist; Ischemia; (Gerbil); Neuroprotection

1. Introduction

Cerebral ischaemia causes a selective pattern of neurodegeneration in man and animals and plays an important role in many clinical situations such as stroke, myocardial infarction and other cardiovascular disorders (Brierley, 1976; Ito et al., 1975; Kirino, 1982). The most vulnerable neurons are found in the hippocampus, striatum and certain layers of the cerebral cortex (Crain et al., 1988). The exact mechanism of ischaemia-induced cell death remains to be elucidated; however, depolarisation causes a large increase in neurotransmitters (glutamate, aspartate, dopamine and serotonin) during ischaemia (Globus et al., 1988; Siesjö, 1992). Glutamate, through an action on *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoazole propionate (AMPA) receptors, allows Ca^{2+} to enter the cell (Choi, 1992; McCulloch, 1992). Ca^{2+} can also enter through voltage-dependent Ca^{2+} channels (VDCC) (Schurr and Rigor, 1992; Siesjö, 1992). The net result of these various mechanisms by which Ca^{2+} concentration is elevated is a Ca^{2+} 'overload' which leads to activation of proteases, nucleases, phospholipases and other degradative enzymes that can lead to free radical production and cell death (Siesjö, 1992).

Many animal models have been used to evaluate the neuropathological changes induced by cerebral ischaemia and whether these changes can be prevented by using neuroprotective agents (Gill et al., 1987; Ginsberg and Busto, 1989; O'Neill et al., 1995a). The Mongolian gerbil (*Meriones unguiculatus*) is unique in having an incomplete circle of Willis, which allows transient global ischaemia to be induced by bilateral carotid artery occlusion (Kirino, 1982; Gill et al., 1987). The CA1 subfield of the hippocampus is particularly susceptible to ischaemia and

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brief periods of ischaemia cause neuronal cell death in this region (Crain et al., 1988; Kirino, 1982; O'Neill et al., 1995b).

Several studies have indicated that many compounds acting at excitatory amino acid receptors have beneficial effects against cerebral ischaemia (Gill et al., 1987; Iversen et al., 1989; Grotta et al., 1990; Sheardown et al., 1990; Bullock et al., 1994). Many investigators have shown that the non-competitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine maleate (MK-801) (Gill et al., 1987, 1988; McCulloch, 1992; Hayward et al., 1993), and competitive NMDA receptor antagonists such as *cis*-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS 19755) (Boast et al., 1988; Grotta et al., 1990; Gotti et al., 1990) and 3-(2-carboxy-piperazine-4yl)-prophenyl-1-phosphonate (D-CPPene) (Boast et al., 1988; Park et al., 1992) are neuroprotective in animal models of global and focal cerebral ischaemia.

The availablility of several synthetic ω-conopeptides such as synthetic MVIIA (SNX-111) has provided the opportunity to evaluate the therapeutic potential of selectively blocking N-type Ca^{2+} channels in a variety of pathological conditions including cerebral ischaemia. SNX-111 has recently been shown to have neuroprotective effects in rat models of focal ischaemia (Smith and Siesjö, 1992; Zhao et al., 1994). Ifenprodil and its derivative [(±)-α-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidineethanol] (SL 82.0715 or eliprodil) are phenylethanolamines which are non-competitive NMDA receptor antagonists and have been shown to have neuroprotective activity in experimental models of ischaemia (Carter et al., 1991; Scatton et al., 1991). However, both of these compounds have also been demonstrated to block voltage-dependent Ca^{2+} channels in cultured rat cortical neurons (Biton et al., 1994) and rat and mouse hippocampal pyramidal neurons in vitro (Church et al., 1994). This ability to block both NMDA receptor mediated responses and Ca^{2+} influx into neurons via VDCC has been forwarded as a possible explanation for the effectiveness of these neuroprotective agents. VDCC have been identified by the use of both pharmacological and biophysical criteria. At least 5 types of high threshold (HVA) and one type of low threshold (LVA) VDCC have been described (reviewed by Tsien et al., 1995). In the studies performed on neuronal VDCC, ifenprodil and eliprodil appear to block multiple subtypes of HVA VDCC (dihydropyridine sensitive L type and non-L type channels). In many neurons, the presence of multiple HVA VDCC makes it difficult to assess the effects on compounds on any one VDCC subtype in isolation. In the present studies, we have examined (1) the ability of these two compounds to block the human N-type VDCC which results from stable expression of $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$ subunits in HEK293 cells and VDCC in acutely isolated cerebellar Purkinje cells which express predominantly P-type VDCC and (2) the effects of ifenprodil and eliprodil administered immediately after occlusion and at 3 and 6 h post-occlusion in the gerbil model of cerebral ischaemia.

2. Materials and methods.

2.1. Electrophysiological methods

2.1.1. Cell culture

Stable transfection of HEK293 cells with human neuronal $\alpha_{1B\text{-}1}$, α_{2b}, and $\beta_{1\text{-}3}$ Ca^{2+} channels subunit expression plasmids (Williams et al., 1992) was performed. 10 cm plates of HEK293 cells were transfected with pcDNA1 $\alpha_{1B\text{-}1}$ (6 μg), pcDNA1 α_{2b} (6 μg), pcDNA $\beta_{1\text{-}3}$ (6 μg) and pMC1neoPoly A (0.25 μg). Transfected cells were selected in HEK293 medium (DMEM, 5% calf serum and Penn./Strep.) containing 500 μg/ml Geneticin (Gibco). Parental cell lines were isolated using cloning cylinders. Parental cell lines that expressed the human $\alpha_{1B\text{-}1}$, α_{2b} and $\beta_{1\text{-}3}$ subunits were identified with a whole cell ^{125}I-ω-CgTx-GVIA binding assay and with $\alpha_{1B\text{-}1}$, α_{2b} and $\beta_{1\text{-}3}$ mRNA analysis. Parental cell lines that bound the highest levels of labeled toxin and expressed all mRNAs were subcloned by limiting dilution. Ligand binding, mRNA and electrophysiological analyses have been used to determine that the selected cell line (G1A1 cells) stably expressed N-type Ca^{2+} channels for 6 months or 50 passages. G1A1 cells were maintained in DMEM (Gibco No. 320-1965AJ) defined, supplemented with bovine calf serum (5.5%) (Hyclone No. A-2151-L), 100 U/ml penicillin G, 100 μg/ml streptomycin (Gibco No. 15140-015) and geneticin (500 μg/ml). One day prior to recording cells were dissociated from the monolayer either mechanically or in Ca^{2+}-free medium. They were then centrifuged and replated onto poly-L-lysine coated glass coverslips in the original medium. Cells were used the following day.

2.1.2. Purkinje cell isolation

Purkinje cells were isolated according to a modification of the method of Mintz et al. (1992). Briefly, the cerebella were isolated from rats aged 6–11 days. Cerebellum vermi were dissected and cut into 0.5–1 mm^3 pieces and incubated at 37°C for 6 min in a solution consisting of 1 mg/ml protease XXIII (Sigma, St. Louis, MO, USA) in 82 mM Na_2SO_4, 30 mM K_2SO_4, 5 mM $MgCl_2$, 2 mM Hepes, 10 mM glucose and 0.01% phenoyl red indicator (pH 7.4). These were then centrifuged and transferred to a solution containing 1 mg/ml BSA (Sigma), 1 mg/ml trypsin inhibitor (Sigma), 82 mM Na_2SO_4, 30 mM K_2SO_4, 5 mM $MgCl_2$, 10 mM Hepes and 10 mM glucose (pH 7.4). Cells were then dissociated by tritiation and plated onto glass coverslips. Purkinje neurons were identified morphologically by their large cell bodies (15–25 μm).

2.1.3. Whole-cell patch clamp

The tight seal whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) was used to record

transmembrane Ca^{2+} currents (I_{Ca}). Coverslips were mounted in a perfusion chamber and thoroughly rinsed with buffer. The majority of G1A1 cells examined were less than 20 μm in diameter and spherical in appearance one day after replating. Currents were recorded using a List EPC-7 or Axopatch 1D amplifier, were filtered by an 8-pole low-pass Bessel filter and stored on computer. Linear leak corrections were performed using a P/N protocol. Series resistance compensation between 40–80% was applied. All experiments were performed at room temperature.

2.1.4. Extracellular solutions

Control buffer solutions were composed of (in mM): NaCl, 138; $CaCl_2$, 5; $MgCl_2$, 1; KCl, 5; Hepes, 10; glucose, 10; pH adjusted to 7.4 with NaOH. Voltage-clamp experiments were performed with cells perfused with solutions containing (in mM): tetraethylammonium chloride (TEACl), 143; $CaCl_2$, 5; $MgCl_2$, 1; Hepes, 10; glucose, 10; pH adjusted to 7.4 with TEA hydroxide.

2.1.5. Pipette solutions

Solutions for voltage-clamp experiments contained (in mM): CsCl, 135; $MgCl_2$, 1; Hepes, 10; diTris phosphocreatinine, 14; MgATP, 3.6; 50 U/ml creatinine phosphokinase; BAPTA, 15, adjusted to pH 7.1 with CsOH.

2.1.6. Materials

All reagents were of the highest commercial grade. ω-CgTx-G*VI*A was from Peninsular Laboratories and ω-Aga-*IV*A from Peptides International (Japan). Ifenprodil was from Tocris Cookson (UK) and eliprodil synthesised at Lilly Research Centre according to the method of Di Fabio et al. (1995). Ifenprodil and eliprodil were made up as stock solutions in DMSO to give final concentrations in solutions which limited the DMSO concentration to ≤ 0.2%.

2.2. Ischaemia methods

2.2.1. Animals and surgery

Male Mongolian gerbils (Bantin and Kingman, Hull, UK) at least 3 months old and weighing in excess of 60 g were used. The animals were maintained in standard lighting conditions and food and water were available ad libitum. The animals were anaesthetised with a 5% halothane/oxygen mixture and maintained using 2% halothane delivered with oxygen at 1 l/min via a face

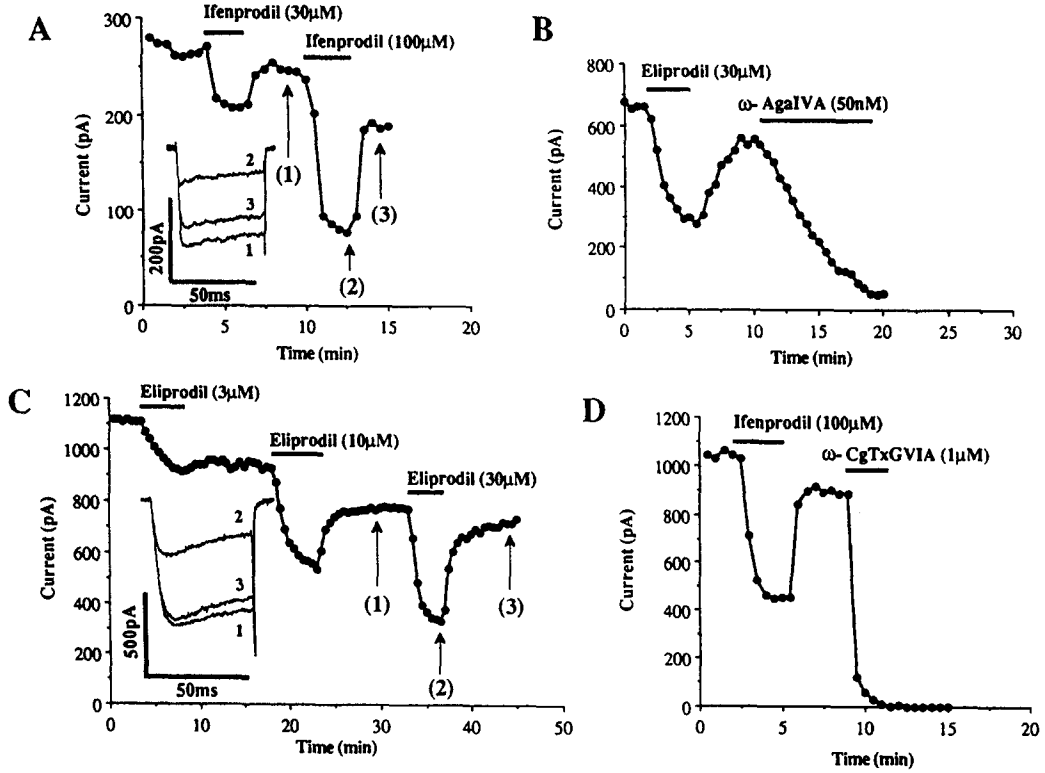


Fig. 1. Representative examples of the concentration- and time-dependent inhibition of N- and P-type Ca^{2+} channels by ifenprodil and eliprodil. (A) Time course of Ca^{2+} currents evoked in cerebellar Purkinje cells from a holding potential (Vh) of −70 mV to a test potential (Vt) of 0 mV (duration 50 ms) every 30 s. Concentration-dependent inhibition of the current by ifenprodil. The inset shows individual current records for the time points indicated: (1) control, (2) during and (3) following washout of ifenprodil. (B) With the same protocol shown in (A), eliprodil (30 μM) inhibition of the whole cell Ca^{2+} current is followed by application of the P-type VDCC specific toxin ω-Aga-IVA (50 nM). (C) Time course of Ca^{2+} currents in HEK293 cells transfected with the human N-type VDCC, Vh = −90 mV, Vt = +10 mV, for 50 ms duration every 30 s. The inset shows individual current traces. (D) Block of the human N-type VDCC by ifenprodil (100 μM) and ω-CgTx GVIA (1 μM).

mask throughout the operation. Through a midline cervical incision, both common carotid arteries were exposed and freed from surrounding connective tissue. In animals to be rendered ischaemic, both common carotid arteries were clamped for 5 min. At the end of the occlusion period blood flow was re-established. In sham operated animals the arteries were exposed but not occluded. The wound was then sutured and the animals allowed to recover. Throughout surgery body temperature was maintained at 37°C using a 'K-TEMP' temperature controller/heating pad (International Market Supply, Cheshire, UK) and a heating lamp. After surgery the animals were placed in a four-compartmental thermacage (Beta Medical and Scientific, UK) which maintained the environmental temperature at 28°C and rectal temperatures were measured for a 6 h period after occlusion. Ifenprodil or eliprodil were administered at 5, 10 or 20 mg/kg i.p. immediately after occlusion followed by 2.5, 5 or 10 mg/kg administered at 3 and 6 h post-occlusion.

2.2.2. Histology

Five days after surgery the animals were perfused transcardially with 30 ml of 0.9% saline followed by 100 ml of 10% buffered formalin solution. The brains were removed and placed in 10% formalin for 3 days, processed and embedded in paraffin wax. 5 μm coronal sections were taken 1.5–1.9 mm caudal to the bregma in the anterior hippocampus using a microtome (Reichert-Jung, Biocut 2035). The slices were stained with haematoxylin and eosin and the neuronal density in the CA1 subfield of the hippocampus was measured using a microscope with grid lines (0.05 × 0.05 mm). The neuronal density is expressed as neuronal density per mm CA1 hippocampus.

2.2.3. Statistics

Statistical analysis of histological data was carried out using ANOVA followed by Student's *t*-test with Bonferroni corrections using $P < 0.05$ as the level of significance.

3. Results

3.1. Electrophysiological results

VDCC were recorded in acutely isolated rat cerebellar Purkinje neurons which have previously been shown to express predominantly P-type VDCC (Regan, 1991; Mintz et al., 1992; Bleakman et al., 1995) and in HEK293 cells transfected with the human N-type VDCC (G1A1 cells) (Williams et al., 1992; Bleakman et al., 1995).

VDCC currents were evoked in Purkinje cells from a holding potential of −70 mV to a test potential of 0 mV every 30 s. Application of either ifenprodil or eliprodil to cells via bath perfusion resulted in a reversible and concentration-dependent inhibition of VDCC current (Fig. 1A and B). Fig. 1B shows a cell in which the reversible block of the VDCC in cerebellar Purkinje neurons by eliprodil (30 μM) was followed by application of the P-type VDCC blocker ω-Aga-IVA (50 nM) which produced almost complete inhibition of the VDCC current.

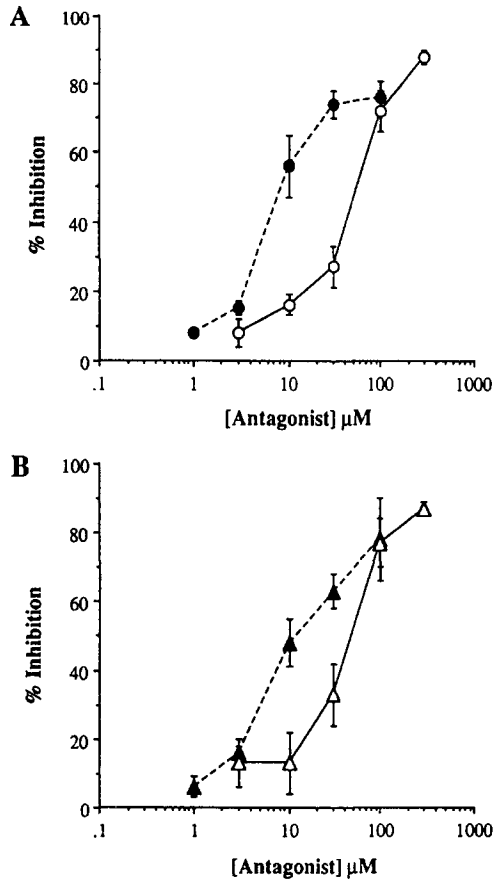
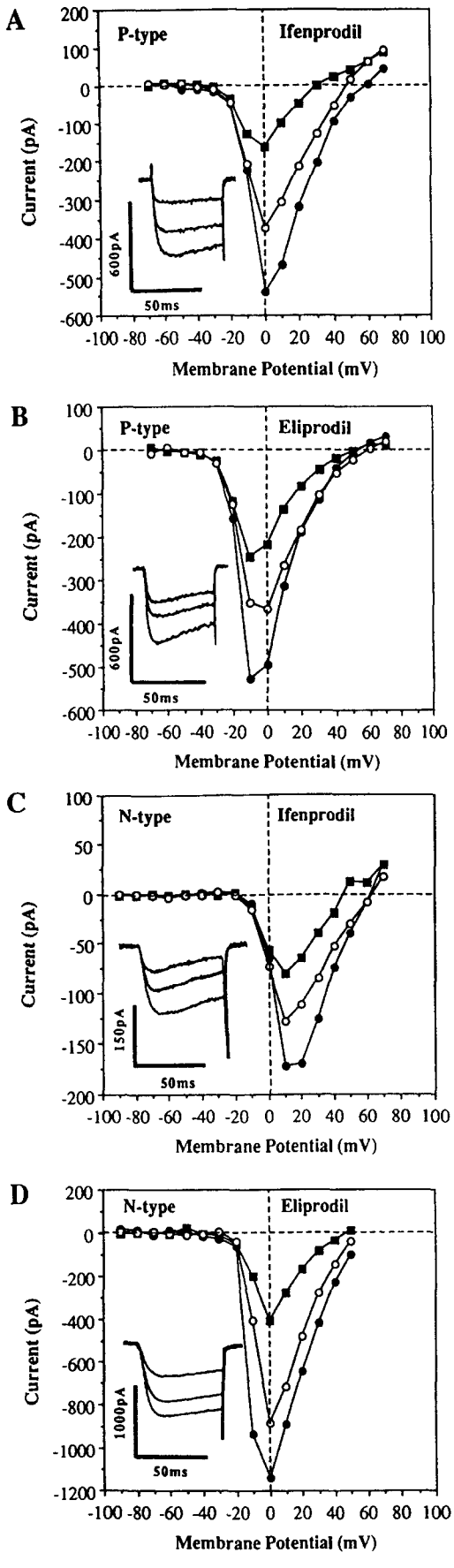


Fig. 2. (A) Concentration-response curves for ifenprodil (○) and eliprodil (●) block of the rat P-type VDCC (B) and ifenprodil (△) and eliprodil (▲) block of the human N-type VDCC. Data points represent mean values ± S.E.M. for between 3 and 8 observations at each concentration.

Currents in G1A1 cells were evoked by depolarizations from a holding potential of −90 mV to a test potential of 10 mV every 30 s (Fig. 1C and D). In a similar manner to the P-type VDCC, ifenprodil and eliprodil produced a reversible and concentration-dependent inhibitions of the VDCC in these cells. Fig. 1D shows the reversible inhibition by ifenprodil (100 μM) followed by application of the N-type VDCC blocker, ω-conotoxin GVIA (1 μM).

Concentration-dependent inhibition curves constructed from cells in which the previous protocol was employed are shown in Fig. 2A and B for the N and P-type VDCC. Steady-state inhibitions were measured and data pooled from the cells in which the compounds were examined. Ifenprodil produced inhibitions of rat P- and human N-type VDCC with approximate IC_{50} values of 60 and 50 μM respectively. IC_{50} values for the block of rat P- and human N-type VDCC by eliprodil were 9 and 10 μM respectively. Complete inhibition of the whole cell Ca^{2+} current



was not observed for either channel type with maximum inhibition of currents for both channel types of approximately 85–90%.

Current-voltage relationships were constructed from currents obtained by depolarizations from a holding potential of −70 mV in cerebellar Purkinje neurons or −90 mV for the N-type VDCC in G1A1 cells to the various test potentials shown. Curves were compared in the absence, presence and following recovery from inhibition by either ifenprodil or eliprodil. The results shown in Fig. 3 illustrate that ifenprodil and eliprodil produce a depression of the currents which differs little in absolute value at any of the test potentials shown. There is also no evidence of any shift in the peak of the current-voltage relationship.

3.2. Ischaemia results

5 μm sections taken 1.5–1.9 mm caudal to the bregma in the anterior hippocampus were examined under a microscope with grid lines. The CA1 pyramidal neurons were found to be degenerated in the 5 min occluded animals. The neuronal death involved nearly all the pyramidal neurons and this neurodegeneration was not evident in any other forebrain region. The pyramidal cell density was counted at three different stereotaxic levels in the CA1 region of the hippocampus and the results expressed as mean ± S.E.M. neuronal density per 1 mm CA1. The results indicated that there was severe loss of neurons in the CA1 region of the hippocampus of 5 min occluded animals.

No changes in rectal temperatures were observed following administration of ifenprodil (Fig. 4A) or eliprodil (Fig. 5A). The lower dose of ifenprodil (5 mg/kg) gave some protection against this ischaemia-induced cell death, while the higher doses doses provided significant neuroprotection (10 and 20 mg/kg) (Fig. 4). The lowest dose of eliprodil gave significant protection at one stereotaxic level, while the higher doses of eliprodil gave significant neuroprotection at all levels. The 10 mg/kg and 20 mg/kg doses of eliprodil gave slightly greater degrees of neuroprotection than the equivalent dose of ifenprodil although the differences in these compounds as neuroprotectants were not significant at these doses (Fig. 4B and Fig. 5B).

Fig. 3. Representative examples of current-voltage relationships for the P- and N-type VDCC. (A) Ifenprodil (100 μM) and (B) eliprodil (30 μM) block of the P-type VDCC, Vh = −70 mV; (C) ifenprodil (100 μM) and (D) eliprodil (30 μM) block of the human N-type VDCC, Vh = −90 mV. Symbols indicate control current-voltage relationships (●), in the presence of drug (■) or following 4 min washout of drug (○). Insets for the figures show individual current records for control currents (lower traces), in the presence of drug (upper traces), and following washout of drug (centre traces), Vt = 0 mV for the P-type VDCC and Vt = + 10 mV for the N-type VDCC. Similar results were obtained in 4 cells for each compound at each channel type.

4. Discussion

The present studies have extended previous studies of the actions of ifenprodil and eliprodil on voltage-dependent Ca^{2+} channels in order to assess the relative potencies of these compounds on defined VDCC subtypes. In previous studies, ifenprodil has been shown to inhibit KCl-induced cGMP increases in cerebellar slices (Carter et al., 1991) and block Ca^{2+} channel mediated responses in vascular smooth muscle (Honda and Sakai, 1987; Honda et al., 1988). More recently, the direct effects of ifenprodil on HVA VDCC in rat and mouse hippocampal pyramidal neurons have been examined. Ifenprodil and eliprodil prevent both KCl-induced increases in $[Ca^{2+}]_i$ and depolarization-induced inward currents carried by barium (I_{Ba}) which appear to be predominantly mediated by L-type VDCC with an IC_{50} value for ifenprodil of approximately 20 μM (Church et al., 1994). However, ifenprodil also prevented KCl-induced increases in $[Ca^{2+}]_i$ in the presence of nimodipine with an IC_{50} value of approximately 13 μM which suggests that ifenprodil can act on at least two types of VDCC in this preparation. The chemical congener of ifenprodil, eliprodil, has been shown to block I_{Ba} in rat cultured cortical neurons with an IC_{50} value of 1.5 μM, an effect which appears to be mediated by both N- and L-type VDCC (Biton et al., 1994). The present studies, which have compared the effects of both ifenprodil and eliprodil on two subtypes of VDCC, have enabled us to make direct comparisons of the relative activity of these two compounds at two VDCC subtypes, the rat P-type VDCC and the human N-type VDCC. For the human N-type VDCC, which is completely inhibited by 1 μM ω-CgTx GVIA,

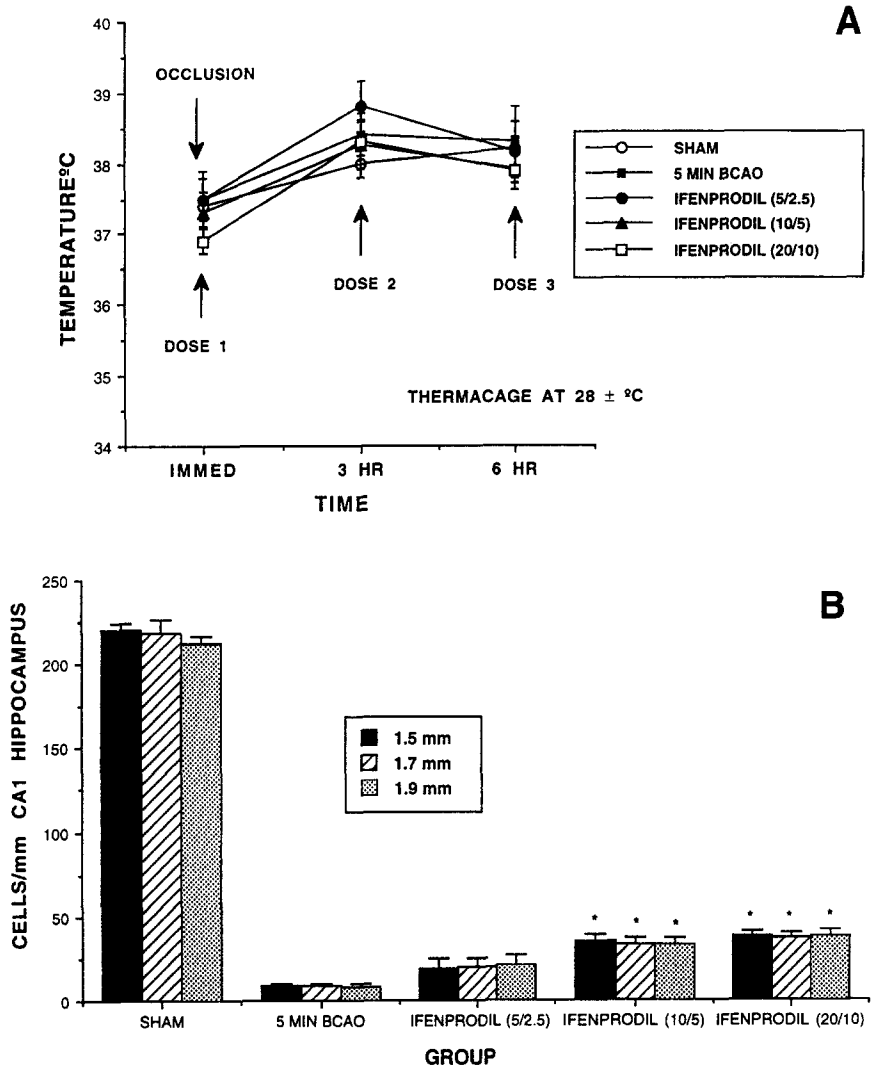


Fig. 4. The effects of ifenprodil administered immediately after surgery followed by two further doses at 3 and 6 h after 5 min bilateral carotid occlusion on rectal temperatures (A) and on the neuronal cell density in the CA1 region of the hippocampus 5 days after surgery (B). Histological results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampal region ($n = 8$ animals per group). 5 min BCAO caused a severe loss in neuronal cells in the CA1 region ($P < 0.0001$). The 5/2.5 mg/kg dose of ifenprodil provided some neuroprotection, while the higher doses gave significant protection against the ischaemia-induced cell death in the hippocampus ($P < 0.05$); Student's t-test with Bonferroni correction factor).

ifenprodil and eliprodil produced current inhibition giving approximate IC_{50} values of 50 and 10 μM respectively. For the VDCC found in cerebellar Purkinje neurons, which is predominantly due to activation of P-type VDCC (90% of whole cell current), similar IC_{50} values were obtained for these two compounds (IC_{50} values of 60 and 9 μM for ifenprodil and eliprodil respectively). The previously reported values for block of I_{Ba} in rat central neurons, which comprise predominantly of L- and N-type VDCC, appear to produce somewhat lower IC_{50} values for ifenprodil (20 μM, Church et al., 1994) and eliprodil (1.5 μM, Biton et al., 1994) than reported in the present study. Other studies have shown that ifenprodil produced a block of the I_{Ba} in hippocampal neurons which did not produce a shift in the current-voltage relationship for the whole cell VDCC (Church et al., 1994). This also appears to be the case for the human N type and rat P type VDCC studies in the present experiments. Thus from the present studies it is clear that eliprodil is more potent than ifenprodil in inhibiting both N- and P-type Ca^{2+} channels. Previous studies on Ca^{2+} channels also suggest that this may be the case for their action at rat N- and L-type Ca^{2+} channel subtypes. Of interest is that there is little difference in the ability of these two compounds to block the steady-state component of NMDA-induced currents with IC_{50} values of 0.75 μM for ifenprodil (Legendre and Westbrook, 1991; Church et al., 1994) and 0.67 μM for eliprodil (Biton et al., 1994).

Bilateral carotid artery occlusion in the gerbil produced

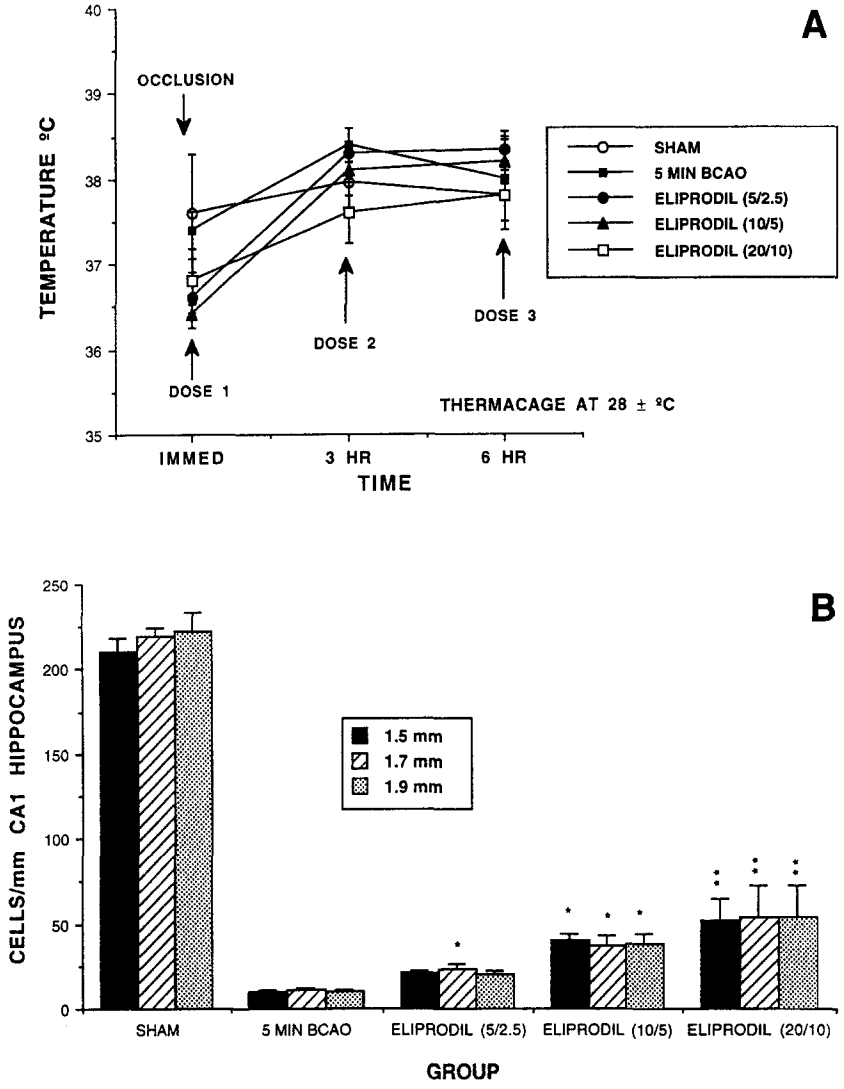


Fig. 5. The effects of eliprodil administered immediately after surgery followed by two further doses at 3 and 6 h after 5 min bilateral carotid occlusion on rectal temperatures (A) and on the neuronal cell density in the CA1 region of the hippocampus 5 days after surgery (B). Histological results are expressed as mean ± S.E.M. viable cells/mm CA1 hippocampal region ($n = 8$ animals per group). 5 min BCAO caused a severe loss in neuronal cells in the CA1 region ($P < 0.0001$). The 5/2.5 mg/kg dose of eliprodil provided some neuroprotection and this reached significance at one stereotaxic level, while the higher doses gave significant protection against the ischaemia-induced cell death in the hippocampus ($P < 0.05$ and $P < 0.01$, respectively); Student's t-test with Bonferroni correction factor).

severe damage to the CA1 neurons of the hippocampus while other regions remained intact. This is in accordance with several studies which have shown selective neurodegeneration following transient forebrain ischaemia in the gerbil (Kirino, 1982; Crain et al., 1988). NMDA receptor antagonists, AMPA receptor antagonists, lipid peroxidation inhibitors and Ca^{2+} antagonists have been able to prevent ischaemic-induced cell death (Gill et al., 1987; Hall et al., 1988; Smith and Meldrum, 1992; Bullock et al., 1994; Gill and Lodge, 1995). Initial studies focused on NMDA receptor antagonists as neuroprotective agents (Gill et al., 1987; McCulloch, 1992). However, the discovery that MK-801 caused hypothermia led to the suggestion that MK-801 was providing neuroprotection by producing hypothermia (Buchan and Pulsinelli, 1990; Corbett et al., 1990) while others postulated that the protective actions of MK-801 are mediated by a small transient hypothermia that acts synergistically with the drug to yield neuroprotection (Hayward et al., 1993). In the present studies we monitored rectal temperatures periodically for 6 h after occlusion and the animals were placed in a maintained warm environment of 28°C. No hypothermic effects were observed with either compound and therefore any protective effects observed are unlikely to be due to hypothermia.

In the present studies we found that ifenprodil administered at the 5 mg/kg dose followed by two 2.5 mg/kg doses gave a small degree of neuroprotection, but this did not reach significance. Both of the higher doses gave significant neuroprotection against the ischaemia-induced cell death; however, the number of cells that survived was similar for both doses examined. The lowest dose of eliprodil also provided some neuroprotection and this reached significance at one stereotaxic level. The 10 mg/kg dose of eliprodil gave similar neuroprotection to the 10 mg/kg of ifenprodil, while the 20 mg/kg dose gave a more pronounced neuroprotection. Other work has shown that ifenprodil reduces the infarct volume following focal ischaemia in the mouse (Gotti et al., 1988, 1990) and Pontecorvo and co-workers (Pontecorvo et al., 1991) reported that ifenprodil increased survival time in a hypoxic environment. Eliprodil (SL 82.0715) also provided protective effects in animal models of cerebral ischaemia and traumatic brain injury (Poignet et al., 1992; Toulmond et al., 1993). Eliprodil has also been reported to have protective effects in a cat model of focal ischaemia (Gotti et al., 1988) and in a mouse model of focal ischaemia (Gotti et al., 1990). Results from Gotti and co-workers (Gotti et al., 1990) indicate that eliprodil was more active than ifenprodil after focal ischaemia in the mouse (58% and 72% protection at 10 mg/kg for ifenprodil and eliprodil respectively). However, in their study both drugs were administered over several days and [^{3}H]PK 11195 binding was used as an indicator of infarct size (Gotti et al., 1990). Previous studies have shown that ifenprodil (45 mg/kg i.p.) gave no protection when given 1 h following a 5 min bilateral carotid artery occlusion in the gerbil whereas NBQX provided significant degrees of protection up to 24 h following occlusion (Sheardown et al., 1993). This result is in good agreement with the small degree of neuroprotection seen with ifenprodil in the present study. We have also observed that the degree of neuroprotection seen with eliprodil and ifenprodil (approximately 20% with 20 mg/kg) is less than observed with either MK-801 (24% at 2 mg/kg) or NBQX (43% at 30 mg/kg) under the same conditions (unpublished observations).

It is interesting to note that both ifenprodil and eliprodil have actions in addition to effects on NMDA receptors and VDCC. For example, ifenprodil has α-adrenergic properties and this may cause vasodilatory effects (Carron et al., 1971; Gotti et al., 1988). Ifenprodil has been reported to cause constriction of isolated pial vessels and this may affect the outcome after an ischaemic insult (Young et al., 1981). Ifenprodil and eliprodil have also been shown to have affinity for σ receptors (Pontecorvo et al., 1991; Poignet et al., 1992), and it has been reported that σ receptor ligands such as BMY 14802 (Contreras et al., 1992), NPC 16377 (Clissold et al., 1993) and JO 1994 (O'Neill et al., 1995a) have neuroprotective actions in animal models of cerebral ischaemia.

Given the potential for multiple sites of action by these compounds, attempts to draw firm conclusions relating their ability to block the two VDCC subtypes to their modest effects on ischaemia are compromised. Our studies indicate that ifenprodil and eliprodil provide a small but significant degree of neuroprotection when administered post-occlusion in the gerbil model of cerebral ischaemia and in addition to their effects on the NMDA receptor complex, both compounds block N- and P-type VDCC.

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