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Acetylcholinesterase Inhibition and Protection by Dizocilpine (MK-801) Enantiomers

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Abstract

The optical isomers of the *N*-methyl-D-aspartate (NMDA) receptor ion-channel blocker dizocilpine (MK-801) were shown to interact with electric eel and rat brain acetylcholinesterase (AChE) in a mixed competitive-noncompetitive way. The (−) form, pharmacologically less active, was the most potent of the two isomers as an AChE inhibitor (K_i for electric eel and rat brain AChE being 6·2 and 17·9 μM, respectively, compared with 200 and 450 μM, respectively, of the (+) form). Both enantiomers premixed with AChE preparations, dose-dependently protected the enzyme from inactivation by diisopropylfluorophosphate (DFP). The maximal protective effects against 40 and 10 μM DFP were in the ranges 10·7–23·8 and 19·5–31·4% of control enzymic activity for the (+) and (−) forms of dizocilpine, respectively. The extent of the protective effect against DFP was increased up to 80·1% of control enzymic activity for (−)-dizocilpine and to 38·4% for (+)-dizocilpine by diluting the enzymic mixtures 1000 times after treatment with the organophosphate agent. The two enantiomers added to AChE 15 min after DFP, failed to reactivate the enzyme. Finally, it was shown that (+)- and (−)-dizocilpine dose-dependently and competitively decreased the DFP bimolecular reaction constant, K_i. We conclude that dizocilpine exerts a protective action towards AChE against irreversible DFP inhibition, but the molecular mechanism of such an action is at present unclear.

Dizocilpine (MK-801), (+)-5-methyl-10,11-dihydro-5H-dibenzo-[*a*, *d*]-cyclohepten-5,10-imine maleate, is a potent anticonvulsant (Clineschmidt et al 1982) and neuroprotective (Goldberg et al 1988) agent. It acts as a noncompetitive antagonist to the *N*-methyl-D-aspartate (NMDA) receptor by blocking the ion channel associated with this receptor (Wong et al 1988). Dizocilpine represents the (+) enantiomer; the corresponding (−) enantiomer is one-fifth as potent as the (+) form as an NMDA receptor antagonist (Wong et al 1988). A number of other channel blockers of the NMDA receptor are known; the dissociative anaesthetic ketamine and the anti-Parkinson drug memantine are other examples of this group of drugs (see Bigge 1991 for review).

Recently, Sparenborg et al (1992) have reported that dizocilpine arrests status epilepticus and prevents brain damage induced by the organophosphorus derivative soman in the guinea-pig. This effect of dizocilpine was attributed to the drug's efficacy in blocking NMDA receptor-mediated spread and maintenance of cholinergically-induced seizure activity. Similarly, the anti-Parkinson drug memantine, which also interacts with the open NMDA receptor ion channel (Kornhuber et al 1989), was reported to be effective prophylactically and therapeutically against soman-induced seizures in the rat (McLean et al 1992). In the case of memantine, however, its anti-soman efficacy was attributed principally to its ability to protect neuronal AChE from inactivation directly (McLean et al 1992). The dissociative anaesthetic ketamine has also been reported to protect AChE from irreversible organophosphate inhibition (Puu 1988).

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The observation that both memantine and ketamine, which are pharmacologically related to dizocilpine, were effective in protecting AChE against organophosphate inhibition, prompted us to examine the possibility that dizocilpine itself or its enantiomer, possessed this type of action.

Materials and Methods

Materials

Acetylcholinesterase (AChE) (EC 3.1.1.7) from electric eel and 5,5'-dithio-bis-(2-nitrobenzoic acid) were purchased from Boehringer Mannheim, Germany. Acetylthiocholine iodide, edrophonium chloride and ethopropazine hydrochloride were from Sigma Chemical Co., MO, USA. Dizocilpine enantiomers were purchased from Research Biochemicals International, MA, USA. Diisopropylfluorophosphate (DFP) was from Fluka, Buchs, Switzerland; a stock solution of this compound was prepared in propylene glycol and stored at 4°C.

A 20% w/v homogenate (glass/Teflon 0·13–0·18 mm clearance) of whole rat (250–300 g, Wistar) brain in 0·05 M sodium phosphate buffer, pH 7·2, was used as a source of brain AChE activity. The final dilution of wet tissue in the assay system was 1 : 300. This preparation hydrolysed on average 6·4 ± 0·8 μmol acetylthiocholine g^{-1} min^{-1}.

Methods

AChE assays. The measurement of AChE was carried out according to the photometric method of Ellman et al (1961) using 0·5 mM acetylthiocholine as substrate in a total

3-mL volume. Ethopropazine (10 μM) was added to brain homogenates to selectively inhibit butyrylcholinesterase.

The IC50 values of (+)- and (−)-dizocilpine were calculated from inhibition curves based on 4 and 6 different concentrations of the compounds, respectively. The data were analysed using the ALLFIT computer program (De Lean et al 1978). The inhibition constants, K_i and K_i', were determined by using three scalar concentrations of (+)- and (−)-dizocilpine and varying the substrate concentration between 0·031 and 0·5 mM. The calculations were based on Lineweaver-Burk plots (Dixon & Webb 1979). All experiments were performed at room temperature (21°C) and the enzymic hydrolysis was started immediately after the addition of inhibitor to the enzyme.

AChE protection. To evaluate AChE protection, fixed 10 μM or 40 μM concentrations of DFP, or buffer alone (controls), were added to aliquots of electric eel AChE (40–60 m units) or rat brain AChE activity (20–30 m units) in 0·25 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) solution, previously mixed with scalar concentrations of the test compounds, or buffer alone (DFP controls), and incubated at room temperature for 15 min in a total 3-mL volume. The residual enzymic activity was then measured by the addition of 0·5 mM acetylthiocholine. In separate experiments, the incubation mixtures containing electric eel AChE, (+)- or (−)-dizocilpine and DFP were diluted 1000 times with 5,5'-dithio-bis-(2-nitrobenzoic acid) solution before assaying enzyme activity. In this case, 2·2 units of eel AChE premixed with the test compounds were incubated with DFP in a total 100-μL volume. Then 10-μL fractions of the incubates were rapidly mixed with 10 mL 5,5'-dithio-bis-(2-nitrobenzoic acid) solution and immediately assayed for enzymic activity. The percent protective effect was calculated according to the equation:

$$\text{Protection (\%)} = \frac{[\text{Inhibition (\%)I}] - [\text{Inhibition (\%)P + I}]}{[\text{Inhibition (\%)I}]} \times 100 \quad (1)$$

where [Inhibition (%)I] and [Inhibition (%)P+I] represent the percent inhibition of enzymic activity following DFP alone or (+)- or (−)-dizocilpine plus DFP, respectively.

DFP inhibition rate. Aliquots of electric eel AChE (0·4–0·5 units) or of crude AChE activity from rat brain (0·25–0·3 units) diluted in 0·25 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) in sodium phosphate buffer, pH 7·2, were mixed at room temperature (21°C) with varying concentrations of (+)- or (−)-dizocilpine, or buffer alone, before the addition of 10 μM DFP. Aliquots of the incubation media were then taken in a time series every second minute in the 2–18 min interval and assayed for progressive enzymic inhibition. The DFP irreversible inhibition constant, k_i, was determined as the rate constant, obtained by linear regression, according to:

$$\ln v = -k_i[I]t + \ln v_0 \quad (2)$$

where v = enzymic activity, t = sampling time and [I] = DFP concentration (Main 1979).

Statistics

The results are presented as means ± s.d. and statistical significance was analysed by means of Wilcoxon matched paired *t*-test (non-parametric) using the NCSS computer program. $P < 0·05$ was considered to be statistically significant.

Results

Inhibition of acetylthiocholine hydrolysis

The two enantiomers of dizocilpine were both inhibitors of AChE. The (−)-form, however, was appreciably more potent than the (+)- form in this action. The mean ± s.d. IC50 values of (−)-dizocilpine were 20 ± 7 and 78 ± 25 μM for electric eel and rat brain AChE, respectively, compared with 0·8 ± 0·1 and > 1 mM, respectively, for (+)-dizocilpine. The inhibitory action of the two compounds was time-independent and immediately reversible upon dilution; when eel AChE aliquots (2 units) premixed with 1 mM (+)-dizocilpine or 0·3 mM (−)-dizocilpine were rapidly diluted 1000 times with 5,5'-dithio-bis-(2-nitrobenzoic acid) solution and immediately assayed for activity, enzymic inhibition decreased from 64 ± 6 to 2 ± 1% and from 92 ± 8 to 3 ± 1% for (+)- and (−)-dizocilpine, respectively.

In saturation experiments performed on the same enzymes, both forms of dizocilpine displayed linear mixed inhibition of acetylthiocholine hydrolysis (Fig. 1). Table 1 shows the competitive, K_i, and the noncompetitive, K_i', inhibition constants of the two enantiomers on eel and rat brain AChE; the competitive character of the inhibitory action appeared to prevail over the noncompetitive one. Finally, dizocilpine isomers were appreciably more potent on eel than on rat brain AChE.

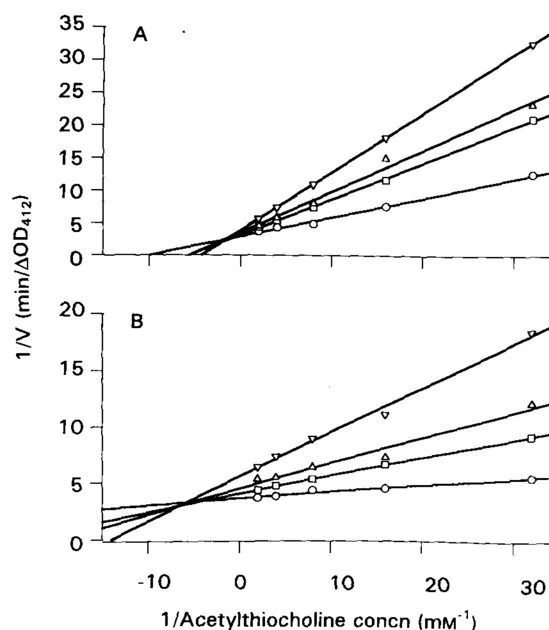

FIG. 1. Double reciprocal plots for inhibition of electric eel AChE by increasing A. (−)-dizocilpine and B. (+)-dizocilpine. Acetylthiocholine in the range 0·031–0·5 mM was used as substrate. A. ○ Buffer, □ 2·5, △ 5, ▽ 10 μM (−)-dizocilpine; B. ○ buffer, □ 0·25, △ 0·5, ▽ 1 mM (+)-dizocilpine.

Table 1. Inhibition constants of (+)- and (−)-dizocilpine towards electric eel and rat brain AChE.

Compound	Eel AChE activity		Brain AChE activity	
	K_i (μM)	K_i' (μM)	K_i (μM)	K_i' (μM)
(+)-Dizocilpine	200 ± 70	1790 ± 282	450 ± 155	2900 ± 1016
(−)-Dizocilpine	6·2 ± 0·9	31 ± 6·7	17·9 ± 4·1	63 ± 17·2

The equilibrium dissociation constants K_i and K_i' were calculated from double reciprocal plots (see Fig. 1), by replotting the slopes and the intercepts on 1/V axis versus three different concentrations of the compounds: the intercepts on baseline gave K_i and K_i', respectively (Dixon & Webb 1979). The conditions of the activity assay were those reported in the legend of Fig. 1. The values are the means ± s.d. of three separate determinations.

Effect of dizocilpine enantiomers on AChE inhibition by DFP

Table 2 shows the effects of increasing (+)-dizocilpine on the inhibition of electric eel and crude rat brain AChE activity by 40 and 10 μM DFP. In the absence of (+)-dizocilpine, these concentrations of DFP inhibited eel AChE almost completely (mean percent inhibition = 95·0 and 94·4, respectively). The addition of (+)-dizocilpine in the range 0·03-1 mM, immediately before DFP, dose-dependently reduced the inhibitory effect of this agent. At 1 mM (+)-dizocilpine, DFP inhibition was 74·8 and 72·3% of control activity against 40 and 10 μM DFP, respectively, corresponding to 21·7 and 23·7% protection, respectively. Similar results were obtained in the crude rat brain homogenate, although the protective effect of (+)-dizocilpine in this preparation was significant at slightly higher concentrations of the drug than in eel AChE. The EC50 values for the protective effect were in the range 0·36–0·45 mM against 40 μM DFP and 0·2–0·41 mM against 10 μM DFP.

Table 3 shows the results obtained in parallel experiments using (−)-dizocilpine as protective agent. The enzymes and the experimental conditions were identical to those used for (+)-dizocilpine. It can be seen that the maximal protective effect of (−)-dizocilpine (31·3 and 31·4% on eel or rat brain AChE, respectively) was similar or just slightly higher than that observed with the (+)-form of the compound. However, this protection developed at drug concentrations markedly lower than those equieffective for (+)-dizocilpine. The EC50 values for this effect were in the range 4–5 and 12–18 μM on eel and brain AChE, respectively. The results in Table 3 also show that AChE protection by (−)-dizocilpine followed a biphasic pattern. It was dose-dependent up to a 30-μM concentration of the drug on eel AChE and 100 μM on rat brain, but it declined rapidly at concentrations higher than these. To ascertain if this effect was due to the direct inhibitory action of dizocilpine towards AChE, which partly masked the protective effect of the drug, experiments were set up in which the enzymic mixtures containing the protective agent and DFP after incubation were diluted 1000 times before being assayed for residual enzymic activity. It was assumed that inhibition by DFP would not be significantly affected by the operation owing to the irreversible character of the inhibitory action of the drug, while inhibition by (+)- or (−)-dizocilpine should be reduced or abolished altogether upon extensive dilution. Table 4 shows the results of these experiments using eel AChE as test enzyme and 40 and 10 μM DFP as inactivating agent. Dilution greatly increased the protective effect of (−)-dizocilpine, which averaged 72·6 and 80·1% of controls against 40 and 10 μM DFP respectively. Under these conditions the protection afforded by (−)-dizocilpine was dose-dependent within the whole concentration range tested. Dilution increased the protective efficacy of (+)-dizocilpine only slightly (28·5 vs 21·7% against 40 μM DFP and 38·4 vs 23·7 against 10 μM DFP). This was expected since (+)-dizocilpine is an extremely weak inhibitor of AChE and therefore its contribution to AChE inhibition had to be small.

In separate experiments it was shown that eel AChE protection by dizocilpine isomers was time-independent. Protection developed as soon as the drugs were added to the enzyme and was not modified by a 15-min preincubation period (data not shown). The action of dizocilpine isomers was purely preventive; these drugs failed to reactivate to any extent the enzyme inhibited by a 15-min preincubation with 40 μM DFP (data not shown).

Lack of protection against edrophonium. In a series of parallel experiments on eel AChE, the protective effect of

Table 2. Effect of increasing concentrations of (+)-dizocilpine on AChE inhibition by DFP.

Treatment	Eel AChE		Rat brain AChE	
	Inhibition (%)	Protection (%)	Inhibition (%)	Protection (%)
Buffer + DFP 40 μM	95·0 ± 7·4	–	99·2 ± 1·0	–
(+)-Dizocilpine 0·03 mM + DFP 40 μM	93·6 ± 8·8	1·6 ± 1·5	98·4 ± 1·7	0·8 ± 0·1
(+)-Dizocilpine 0·1 mM + DFP 40 μM	90·9 ± 11·0*	4·6 ± 3·9	97·9 ± 1·9	1·3 ± 1·2
(+)-Dizocilpine 0·3 mM + DFP 40 μM	85·8 ± 14·4*	10·5 ± 8·9	95·5 ± 2·5*	3·6 ± 2·4
(+)-Dizocilpine 1 mM + DFP 40 μM	74·8 ± 8·5*	21·7 ± 4·2	88·4 ± 8·2*	10·7 ± 7·9
Buffer + DFP 10 μM	94·4 ± 7·6	–	98·2 ± 4·0	–
(+)-Dizocilpine 0·03 mM + DFP 10 μM	93·6 ± 7·0	0·8 ± 0·6	98·2 ± 3·6	0·1 ± 0·2
(+)-Dizocilpine 0·1 mM + DFP 10 μM	87·8 ± 9·3*	7·4 ± 4·1	96·4 ± 5·0	2·1 ± 1·5
(+)-Dizocilpine 0·3 mM + DFP 10 μM	81·3 ± 9·5*	14·0 ± 5·1	91·9 ± 2·6*	6·6 ± 2·9
(+)-Dizocilpine 1 mM + DFP 10 μM	72·3 ± 7·2*	23·7 ± 5·2	75·1 ± 6·9*	23·8 ± 4·1

Aliquots of eel AChE (40–60 m units) or crude rat brain AChE activity (20–30 m units) were mixed with the indicated concentrations of (+)-dizocilpine immediately before the addition of 40 or 10 μM DFP. The samples were then incubated for 15 min at room temperature (21°C) and assayed for enzymic activity. The percent inhibition was calculated vs the controls with buffer alone. The percent protection was calculated according to the equation shown in Materials and Methods. The values are the means ± s.d. of six separate experiments.
*$P < 0·05$ Wilcoxon matched paired test vs respective buffer + DFP value.

Table 3. Effect of increasing concentrations of (−)-dizocilpine on AChE inhibition by DFP.

Treatment	Eel AChE		Rat brain AChE	
	Inhibition (%)	Protection (%)	Inhibition (%)	Protection (%)
Buffer + DFP 40 μM	97·7 ± 1·0	–	98·8 ± 0·7	–
(−)-Dizocilpine 3 μM + DFP 40 μM	90·4 ± 13·2	7·8 ± 6·0	97·6 ± 0·7	1·2 ± 1·1
(−)-Dizocilpine 10 μM + DFP 40 μM	78·0 ± 10·5*	20·5 ± 8·5	95·2 ± 2·6*	3·6 ± 2·4
(−)-Dizocilpine 30 μM + DFP 40 μM	72·5 ± 5·3*	25·7 ± 4·6	85·2 ± 10·0*	13·7 ± 9·6
(−)-Dizocilpine 100 μM + DFP 40 μM	83·4 ± 1·0*	15·1 ± 0·3	79·3 ± 6·0*	19·5 ± 5·3
(−)-Dizocilpine 300 μM + DFP 40 μM	93·0 ± 2·3	4·7 ± 1·5	87·1 ± 8·2*	11·5 ± 7·5
Buffer + DFP 10 μM	89·6 ± 11·6	–	96·7 ± 2·1	–
(−)-Dizocilpine 3 μM + DFP 10 μM	81·7 ± 4·7	8·8 ± 5·0	96·2 ± 4·0	0·5 ± 0·3
(−)-Dizocilpine 10 μM + DFP 10 μM	61·8 ± 6·0*	31·3 ± 6·4	81·6 ± 4·3*	15·7 ± 4·1
(−)-Dizocilpine 30 μM + DFP 10 μM	61·9 ± 7·6*	31·2 ± 7·1	73·2 ± 5·2*	24·5 ± 5·2
(−)-Dizocilpine 100 μM + DFP 10 μM	81·7 ± 6·1	8·9 ± 5·9	66·3 ± 6·1*	31·4 ± 5·1
(−)-Dizocilpine 300 μM + DFP 10 μM	84·6 ± 5·2	5·7 ± 4·3	78·5 ± 3·7*	19·1 ± 3·6

The values are the means ± s.d. of six separate experiments. Other details are as in Table 2.
$*P < 0.05$ Wilcoxon matched paired t-test vs respective buffer + DFP value.

dizocilpine isomers was tested against the competitive reversible AChE inhibitor edrophonium (Taylor & Lappi 1975). This drug was used at the fixed 30-μM concentration. At this concentration edrophonium caused a mean 97 ± 2% inhibition of the enzyme. Neither (+)-dizocilpine, in the range 0·03–1 mM, nor (−)-dizocilpine, in the range 0·01–0·3 mM, caused an appreciable decrease in the inhibitory action of edrophonium (data not shown).

Effect on DFP inhibition rate. Table 5 shows the effect of increasing concentrations of the two isomers of dizocilpine on DFP bimolecular reaction constant, k_i, on eel and rat brain AChE. As expected from the inhibition constants of Table 1, the (−)-isomer was more efficient than the (+)-isomer in preventing irreversible inhibition by DFP. The results obtained can be explained by a simple interaction at the active site since they can be described by the equation:

$$k_{obs} = k_i/(1 + [\text{dizocilpine}]/K_i). \quad (3)$$

According to this equation a 50% reduction in k_i is thus obtained when the concentration of dizocilpine equals its K_i value. This point was generally fulfilled although the observed constants for (−)-dizocilpine on eel AChE were slightly lower than calculated.

Discussion

This work provides evidence that the NMDA receptor ion-channel blocker dizocilpine exerts a dose-dependent protective action towards AChE. This was revealed by a decrease in the inhibitory action of the organophosphate DFP in the presence of dizocilpine. Such an effect is stereoselective since the (−)-form of the drug, pharmacologically less active (Wong et al 1988), is considerably more potent than the (+)-form as AChE protector. This correlates well with the affinity of the two isomers for AChE. The observed effect is purely preventive since dizocilpine does not reactivate the enzyme previously inhibited by DFP to any extent.

Several purely reversible inhibitors of AChE have been previously reported to protect this enzyme from organophosphate-induced inactivation (Wills 1970). Meptazinol (Galli & Mazri 1988), tacrine (Wu & Yang 1989; Galli &

Table 4. Protection of eel AChE by increasing (+) and (−)-dizocilpine from irreversible inhibition by 40 and 10 μM DFP following extensive dilution.

Pretreatment	40 μM DFP		10 μM DFP	
	Inhibition (%)	Protection (%)	Inhibition (%)	Protection (%)
Buffer	98·7 ± 1·5	–	95·2 ± 3·6	–
(+)-Dizocilpine 30 μM	97·7 ± 3·0	1·2 ± 1·5	92·6 ± 6·1	2·8 ± 2·6
(+)-Dizocilpine 100 μM	93·3 ± 5·6*	5·4 ± 4·3	89·0 ± 5·7*	6·5 ± 3·2
(+)-Dizocilpine 300 μM	92·6 ± 4·3*	6·1 ± 3·1	75·3 ± 9·7*	21·4 ± 8·4
(+)-Dizocilpine 1 mM	70·2 ± 3·3*	28·5 ± 2·8	59·2 ± 12·5*	38·4 ± 12·2
Buffer	98·1 ± 2·5	–	93·5 ± 7·0	–
(−)-Dizocilpine 3 μM	97·4 ± 2·4	1·1 ± 1·6	83·5 ± 13·0*	10·1 ± 7·8
(−)-Dizocilpine 10 μM	79·4 ± 16·0*	19·2 ± 14·0	62·0 ± 20·1*	27·5 ± 22·5
(−)-Dizocilpine 30 μM	63·7 ± 22·0*	35·2 ± 21·5	38·2 ± 12·0*	57·4 ± 9·5
(−)-Dizocilpine 100 μM	38·6 ± 18·1*	60·5 ± 17·6	23·5 ± 12·7*	73·3 ± 12·4
(−)-Dizocilpine 300 μM	34·3 ± 11·7*	64·7 ± 11·6	18·5 ± 7·9*	78·3 ± 9·6
(−)-Dizocilpine 1 mM	26·4 ± 7·4*	72·6 ± 7·5	15·0 ± 1·6*	80·1 ± 5·9

Aliquots (0·8–1·0 units) of electric eel AChE premixed with the indicated concentrations of (+)- and (−)-dizocilpine were incubated at room temperature (21°C) for 15 min with 40 or 10 μM DFP. The samples were then diluted 1000 times with 0·25 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) solution and assayed immediately for enzymic activity. The values are the means ± s.d. of six separate experiments.
$*P < 0.05$ Wilcoxon matched paired t-test vs respective buffer.

Table 5. The effect of increasing (+) and (−)-dizocilpine on inhibition of electric eel and rat brain AChE by DFP.

Pretreatment	Eel AChE k_i DFP (10^3 M^{-1} min^{-1})	Brain AChE activity k_i DFP (10^3 M^{-1} min^{-1})
Buffer	13·6 ± 3·7	20·2 ± 5·9
(+)-Dizocilpine 10 μM	12·9 ± 3·0	18·4 ± 6·7
(+)-Dizocilpine 30 μM	11·9 ± 1·5	17·7 ± 8·2
(+)-Dizocilpine 100 μM	8·9 ± 0·8	15·7 ± 5·7
(+)-Dizocilpine 300 μM	5·3 ± 0·6	11·7 ± 2·9
(+)-Dizocilpine 1 mM	1·6 ± 1·4	7·4 ± 2·4
(−)-Dizocilpine 1 μM	12·5 ± 1·0	19·8 ± 2·4
(−)-Dizocilpine 3 μM	7·25 ± 2·9	17·2 ± 1·4
(−)-Dizocilpine 10 μM	4·35 ± 0·6	14·7 ± 1·3
(−)-Dizocilpine 30 μM	1·05 ± 0·4	7·4 ± 2·4
(−)-Dizocilpine 100 μM	0·85 ± 0·2	3·3 ± 3·2

Aliquots of electric eel AChE (0·4–0·5 units) or of crude AChE activity from rat brain (0·25–0·3 units) taken up with 0·25 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) in sodium phosphate buffer, pH 7·2, at room temperature (21°C), were pretreated with the indicated concentrations of (+)- or (−)-dizocilpine immediately before the addition of 10 μM DFP. Aliquots of the incubation media were then taken in a time series every second minute in the interval 2–18 min and assayed for residual enzymic activity. The DFP irreversible inhibition constant, k_i, was determined according to the equation shown in Materials and Methods. Values are the means ± s.d. of three separate determinations.

Mori 1991) and E2020 (Galli et al 1994) are examples of this type of compound. These agents possess a high affinity for the enzyme and their protective action is generally explained by assuming that these inhibitors prevent AChE phosphorylation by shielding the active site of the enzyme from organophosphate derivatives (Berman & Leonard 1990; Galli et al 1992). The protective action of these compounds can be observed only after their removal from the incubation medium, since otherwise their own inhibitory action would mask the effect. This mechanism of action is also probably valid in the case of dizocilpine isomers; the observation that they decrease the DFP inhibition rate in a substantially competitive way, strengthens this point. These drugs, however, are endowed with relatively low affinities for AChE. Their protective effects do not require removal of the drugs to appear, although this operation may enhance the effect. Therefore, it seems that in the protective mechanism of these compounds there is a component of action other than simple competition for the active site of the enzyme, which contributes to the overall protective effect of the drugs. This point is also supported by the following observations. The protective action of dizocilpine isomers shows analogies with those of memantine (McLean et al 1992), which is devoid of anticholinesterase activity, and of ketamine (Puu 1988), which is a weak inhibitor of AChE (Puu et al 1991). Dizocilpine, like memantine (McLean et al 1992), does not protect AChE against edrophonium, which directly interacts with the active site of the enzyme (Wilson & Quan 1958; Taylor & Lappi 1975).

Our data do not permit us to establish the mechanism of the protective action of dizocilpine. However, it may be hypothesized that dizocilpine isomers interact with a site on the enzyme, other than the active site, thus preventing or modifying binding of DFP. Alternatively, the binding of dizocilpine might induce a modification in the enzyme structure unfavourable to its phosphorylation by DFP. However, other mechanisms are possible. The possibility that dizocilpine might slow the aging of the phosphorylated AChE, as hypothesized by Puu (1988) for the protective effect of ketamine against sarin, seems untenable since the aging half-life for AChE phosphorylated by DFP is reported to be 4·6 h (Hobbiger 1956), that is, a time interval considerably longer than the 15-min incubation times used in this work.

Sparenborg et al (1992) have reported that (+)-dizocilpine, in the 0·5–5·0 mg kg^{-1} dose range, is effective as an anticonvulsant and neuroprotectant against soman-induced seizures and brain damage in the guinea-pig. At these doses a direct protective effect of the drug on neuronal AChE appears unlikely; the anti-soman action of dizocilpine appears to be better explained by the NMDA receptor ion channel-blocking properties of the drug.

In summary, the optical isomers of dizocilpine are shown to protect AChE in-vitro from the irreversible inhibition induced by DFP. A further investigation is now necessary to establish if this effect is also detectable in-vivo and if it can be utilized to prevent the effects of organophosphate poisoning.

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