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In-vitro and in-vivo protection of acetylcholinesterase by eseroline against inactivation by diisopropyl fluorophosphate and carbamates

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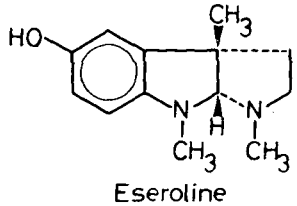
The protective action of eseroline—(3aS,8aR)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol—salicylate against (DFP) diisopropyl fluorophosphate and carbamate poisoning of cholinesterases (ChEs) has been examined in-vitro with human erythrocytes and purified preparations of electric eel acetylcholinesterase (AChE) and of horse serum butyrylcholinesterase (BuChE), and in-vivo using mice. Eseroline afforded 50% protection (ED 50) of erythrocyte AChE against inactivation by 1 μM DFP, physostigmine or neostigmine, at concentrations of 4·3, 22 and 23·5 μM, respectively, while for eel AChE protection against 10 and 30 μM DFP, 0·3 and 1 μM physostigmine and 1 μM neostigmine the eseroline ED 50 values were 0·3, 0·4, 0·7, 1·9 and 5·6 μM, respectively. On the other hand, up to 0·3 mM eseroline did not appreciably affect the inhibitory action of the same drugs on horse serum BuChE. Eseroline concentrations in the range 0·1–1 mM were able to reactivate 20–42% of erythrocyte AChE previously inhibited by 100 μM physostigmine, but failed to reactivate the DFP (10 μM)-pretreated enzyme to any extent. Finally, eseroline salicylate injected into mice (10 mg kg^{-1} s.c.) protected an average of 82 and 26% of the animals against lethal doses of DFP (7 mg kg^{-1} s.c.) and physostigmine sulphate (1 mg kg^{-1} i.p.) respectively, which were administered 15 min later. These results indicate that the protective activity of eseroline correlates well with its own anti-ChE profile, and that the effectiveness of the protection depends largely on the rate of AChE inhibition by the agents used to inactivate the enzyme.

It is now well established that short-lasting cholinesterase (ChE) inhibitors may protect these enzymes against inactivation by irreversible inhibitors (Koelle 1946; Bergmann & Shimoni 1952; Depierre & Martin 1958; Lüllmann et al 1967; Schoene et al 1976; Deyi et al 1981; Ashani et al 1983). This type of action is of interest in many respects, but particularly with a view to developing drugs which are potentially effective against poisoning by toxic anti-ChE agents (Koster 1946; Berry & Davies 1970; Schoene et al 1976; Gordon et al 1978; Dirnhuber et al 1979; Ashani et al 1983).

We recently demonstrated that eseroline—(3aS,8aR)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol—the polycyclic aminophenol derived from physostigmine by hydrolysis of the *N*-methylcarbamyl group, is endowed with remarkable inhibitory activity on AChE from various sources (Galli et al 1982). However, unlike physostigmine and carbamates in general, which inhibit ChE with a progressive action (Augustinsson & Nachmansohn 1949; Main & Hastings 1966) and which allow only a very slow regeneration of the free active enzyme from the carbamoylated derivative (Wilson et al 1960), AChE inhibition by eseroline develops instantaneously and is promptly reversible on dilution (Galli et al 1982).



Eseroline

Eseroline injected into laboratory animals induces opiate receptor-mediated analgesia as well as other interesting central effects (Bartolini et al 1978, 1981, 1982; Galli et al 1979; Jhamandas et al 1981; Fürst et al 1982). Surprisingly, however, eseroline does not give rise to any symptomatology of cholinergic stimulation even at several times higher doses than those inducing analgesia (Bartolini et al 1978; Galli et al 1982). This lack of cholinomimetic effects with eseroline, which clearly differentiates it from physostigmine, had already been reported much previously (Stedman & Barger 1925; Schweitzer et al 1939) and

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had come to represent a firmly-established point in the structure-activity relationship studies of anti-ChE agents (Stempel & Aeschlimann 1956; Long 1963).

These findings seemed to us very promising with a view to a potentially effective protective action of eseroline on AChE against irreversible inactivation by toxic anti-ChE agents; hence the present study.

A preliminary report of this work was presented at the 2nd International Meeting on Cholinesterases, Bled, Yugoslavia, September 1983.

MATERIALS AND METHODS

Reagents and drugs. 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCh) and butyrylthiocholine iodide (BuTCh) were purchased from Boehringer Mannheim Gmbh (FRG). Physostigmine sulphate and neostigmine bromide were purchased from Sigma Chemical Co. (St Louis, MO, USA). Diisopropyl fluorophosphate (DFP) was from Fluka AG (Buchs, Switzerland) and morphine hydrochloride from Carlo Erba (Milan, Italy). Eseroline free base and eseroline salicylate were synthesized according to Galli et al (1979).

Cholinesterases. Purified AChE (1000 units mg^{-1} lyophilized material) from *Electrophorus electricus* was purchased from Boehringer Mannheim Gmbh and purified horse serum BuChE (13·3 units mg^{-1} protein) from Sigma Chemical Co. Human erythrocyte AChE activity was obtained from 0·1 ml heparinized blood. RBCs were washed 3 times by centrifugation with 9 ml of saline solution and resuspended in 20 ml of 50 mM sodium phosphate buffer, pH 7·2 in saline.

Cholinesterase activity was measured at 25 °C and pH 7·2 by the photometric method of Ellman et al (1961) using 0·25 mM DTNB in 50 mM sodium phosphate buffer as chromogenic reagent and 0·5 mM ATCh, or 1 mM BuTCh, as substrates in a total 3 ml volume. The variations in optical absorbance at 412 nm were measured at 30 and 60 s intervals by means of a Beckman, Acta III, spectrophotometer equipped with a chart recorder. Concentration values refer to final concentrations in the activity assay.

Inhibition experiments. 0·9 ml aliquots of washed human erythrocyte suspensions (0·015–0·020 units of AChE), or 50 µl aliquots of purified electric eel AChE solutions in buffer (1·25 units ml^{-1}), were brought up to 2·85 ml with 0·37 mM, or 0·27 mM, DTNB, respectively, and preincubated at 25 °C for 30 min with 6–8 graduated concentrations of eseroline salicylate, or buffer alone (controls), before being assayed for enzymic activity as described earlier.

Protection experiments on erythrocyte AChE. Graduated concentrations of eseroline salicylate and buffer alone (50 µl) were added to 0·9 ml aliquots of washed RBCs. Immediately after mixing, an appropriate fixed amount of the test irreversible inhibitor, or buffer alone (activity controls), was added to all samples in 50 µl volume. The mixtures were then incubated at 25 °C for 30 min. Erythrocytes were separated by centrifuging at low speed for 5 min, rapidly resuspended in 1 ml of buffer in saline and centrifuged again. The pellets were resuspended in 0·9 ml buffer and assayed for enzymic activity. The washing of erythrocytes and the measurement of hydrolysis velocity were accomplished within 15 min. The inhibition caused by eseroline alone (eseroline blanks) was less than 3% and 6% for 0·1 and 1 mM eseroline salicylate, respectively, and it was not taken into account in the calculation of results. The drug concentration values in these experiments refer to final concentrations in the 1 ml incubation volume.

Protection experiments on electric eel AChE. Appropriate fixed amounts of test inhibitors, or buffer alone, were added to aliquots of eel AChE, previously mixed with graduated concentrations of eseroline salicylate, or buffer alone, and incubated at 25 °C for 30 min. At the end of the incubation time, 50 µl fractions of the mixtures, corresponding to 0·050–0·070 units of enzymic activity, were diluted rapidly 500 times with 0·27 mM DTNB in 50 mM phosphate buffer, pH 7·2, and immediately assayed for AChE activity in the presence of 0·5 mM ATCh. The dilution of the samples and the measuring of reaction velocity were accomplished in less than 2 min. Under these conditions, eseroline alone caused an average of 3 and 5% inhibition of AChE at 3 and 10 µM drug concentrations, respectively. The drug concentration values in these experiments refer to final concentrations in the incubation mixtures, before dilution of the samples.

In-vivo protection experiments. Groups of 10 randomized Swiss mice (25–28 g) were treated s.c. with eseroline salicylate or with saline (controls) before the injection of lethal doses of physostigmine sulphate (1 mg kg^{-1} i.p.) or DFP (7 mg kg^{-1} s.c.). Lethality was assessed 90 min later, although the animals were controlled for an additional 24 h.

Calculation of results. The percent protection was calculated according to the equation:

$$\text{Protection (\%)} = \frac{[\text{Inhibition (\%)}_I] - [\text{Inhibition (\%)}_{E+I}]}{[\text{Inhibition (\%)}_I]} 100$$

where [Inhibition $(\%)_I$] and [Inhibition $(\%)_{E+I}$] represent the percent inhibition of enzymic activity, or the percent lethality in the in-vivo experiments, following inhibitor alone or eseroline plus inhibitor, respectively.

RESULTS

In-vitro protection experiments.

Eseroline is itself a remarkable inhibitor of AChE (Galli et al 1982); to evaluate the protective action of the drug on this enzyme eseroline therefore had to be removed as completely as possible from the incubation medium before measurement of enzymic activity. In the experiments with erythrocytes, this was achieved by centrifuging and washing the erythrocytes which had been preincubated with eseroline and inhibitors, before assaying residual AChE activity. In the case of electric eel AChE and of horse serum BuChE, which were used in soluble forms, the inhibitory effects due to eseroline itself were minimized by rapidly diluting samples 500 times before measuring enzymic activity. It was assumed that if these operations—i.e. washing away or diluting drug excess—were carried out reasonably rapidly, the enzymic inactivation by DFP, physostigmine and neostigmine would not be significantly affected by the treatment, owing to the basically irreversible character of the inhibitory actions of these drugs (Wilson et al 1960).

The results of Fig. 1 show the protective effects on erythrocyte AChE of increasing concentrations of eseroline against fixed highly inhibitory concentrations of DFP, physostigmine and neostigmine. To enable a direct comparison with the inhibitory effect of the drug to be made, the inhibition curve of eseroline on this enzyme has also been included in the same Fig. The experimental data show that eseroline protected AChE against inactivation by all the tested inhibitors. This action was dose-dependent and showed a pattern of progress which closely resembled that for inhibition. The protection of erythrocyte AChE by eseroline was markedly more effective against DFP than against physostigmine and neostigmine, the ED50 for such an action (calculated according to Litchfield & Wilcoxon 1949; 95% confidence limits) being 4·3 (2·2–8·3), 22 (12·7–38) and 23·5 (14·3–38·6) μM, respectively. Under the same experimental conditions eseroline IC50 was 3·4 (1·6–7·2) μM. In the absence of eseroline, 1 μM DFP, physostigmine or neostigmine inhibited on average 93, 78 and 81% of enzymic activity, respectively. Although the mean protection values against physostigmine were slightly higher than those against neostigmine, the difference was not significant.

The results obtained on electric eel AChE are shown in Fig. 2. Eseroline ED50 values for the protection of this enzyme were 0·3 (0·1–0·7), 0·4

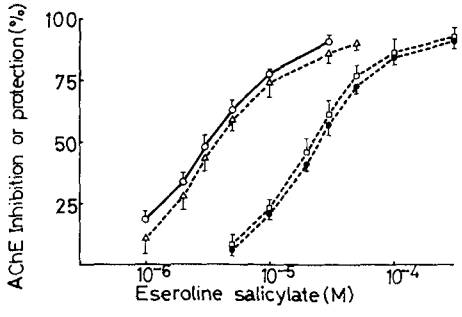

FIG. 1. Inhibition of human erythrocyte AChE by eseroline and its protection by the same drug against various anti-ChE agents. The experimental procedure is described in Materials and methods. AChE inhibition (○——○); AChE protection against 1 μM DFP (△ - - - - △), 1 μM physostigmine (□ - - - - □), 1 μM neostigmine (● - - - - ●). Inhibition and protection (- - -) values are expressed as percentages of uninhibited AChE activity of their respective controls. The points on the graph represent the mean ± s.e.m. of 3–5 separate experiments performed in duplicate.

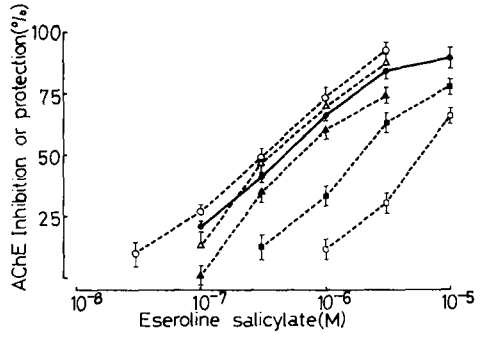

FIG. 2. Inhibition of electric eel AChE by eseroline and its protection by the same drug against various anti-ChE agents. The experimental procedures are described in Materials and methods. AChE inhibition (●——●); AChE protection against 10 μM (○ - - - - ○) and 30 μM (△ - - - - △) DFP, 0·3 μM (▲ - - - - ▲) and 1 μM (■ - - - - ■) physostigmine and 1 μM neostigmine (□ - - - - □). Inhibition and protection (- - -) values are expressed as percentages of uninhibited enzymic activity of their respective controls. The points on the graph represent the mean ± s.e.m. of 3–4 separate experiments performed in duplicate.

(0·1–1·3), 0·7 (0·2–1·8), 1·9 (0·8–4·5) and 5·6 (2·1–14·9) μM against 10 and 30 μM DFP, 0·3 and 1 μM physostigmine and 1 μM neostigmine, respectively. Eseroline IC50 was in this case 0·4 (0·1–1·1) μM. The mean percent inhibition of the enzyme by 10 and 30 μM DFP, 0·3 and 1 μM physostigmine and 1 μM neostigmine in the absence of eseroline was 86, 98, 96, 98 and 98%, respectively. In this system therefore both inhibition and protection by eseroline developed at lower concentrations of the drug than those necessary for erythrocyte AChE. However, as in the case of erythrocyte AChE, eseroline was more effective in protecting eel AChE against DFP than against the two carbamates; eel AChE protection against 10 and 30 μM DFP in fact occurred at equal eseroline concentrations to those necessary for inhibition of the enzyme, or even lower ones. The protective action of eseroline against DFP or physostigmine was clearly dependent on the concentrations of these inhibitors. This concentration effect was, however, more evident for physostigmine than for DFP. Finally, in the case of eel enzyme, eseroline was seen to be clearly more effective against physostigmine than against neostigmine.

In the experiments so far examined, eseroline was added to the enzyme immediately before the irreversible inhibitor. In a series of separate experiments, eseroline (0·1 mM) was added to erythrocytes at different times after the addition of inhibitory concentrations of physostigmine (1 μM) or DFP (2 μM). In all cases, protection of enzymic activity was lower than that observed when the addition of eseroline preceded that of the irreversible inhibitor (data not shown).

Unlike AChE, eseroline was practically ineffective in protecting horse serum BuChE against inactivation by the same inhibitors. As reported in Table 1, maximal 0·3 mM concentrations of eseroline protected on average only 11, 12 and 1% of BuChE activity against 0·3 μM physostigmine, 0·6 μM neostigmine and 0·1 μM DFP, respectively. The use of eseroline concentrations exceeding 0·3 mM was avoided to minimize the formation of its oxidation product, rubreserine (Galli et al 1982), which is a BuChE inhibitor and might therefore have interfered with the protective effects of eseroline.

AChE reactivation experiments

To verify whether eseroline, besides protecting AChE, was also able to reactivate it, the restoration of enzymic activity by increasing eseroline was investigated in human erythrocytes which had been extensively pretreated with high concentrations of physostigmine (100 μM) or DFP (10 μM). As appears from the data of Table 2, eseroline failed to reactivate AChE inhibited by DFP to any extent. In other words, eseroline proved unable to induce the

Table 1. Effect of eseroline on the inhibition of BuChE by different anti-ChE agents. Inhibitors were added to aliquots of horse serum BuChE previously mixed with 0·3 mM eseroline salicylate or buffer alone. The mixtures were then incubated at 25 °C for 30 min. 50 μl fractions of the samples corresponding to 0·05 units of BuChE were rapidly diluted 500 times with 0·27 mM DTNB in 50 mM sodium phosphate buffer, pH 7·2, and immediately assayed for enzymic activity in the presence of 1 mM BuTCh as substrate. The concentration values of the drugs refer to the final concentrations present in the incubation mixtures before dilution. Values represent the means of two separate experiments performed in duplicate.

	Inhibition (%)	Protection (%)
Buffer + physostigmine 0·3 μM	98	
Eseroline 0·3 mM + physostigmine 0·3 μM	87	11
Buffer + neostigmine 0·6 μM	96	
Eseroline 0·3 mM + neostigmine 0·6 μM	84	12
Buffer + DFP 0·1 μM	98	
Eseroline 0·3 mM + DFP 0·1 μM	97	1

Table 2. Reactivation by eseroline of physostigmine- and DFP-inhibited erythrocyte AChE. Aliquots of washed human erythrocytes preincubated with 100 μM physostigmine, or with 10 μM DFP, or with saline alone (enzymic activity controls), at 25 °C for 60 min, were separated from the incubation media by centrifugation and resuspended in 1 ml of eseroline salicylate at the indicated concentrations or of saline alone (saline controls). After 1 min (physostigmine-pretreated erythrocytes) or 10 min (DFP-pretreated erythrocytes) incubations at 25 °C, erythrocytes were again centrifuged, washed with saline and pelleted again before being resuspended in buffer, 0·9% w/v NaCl, (0·9 ml) and assayed for AChE activity. Reactivation (%) = 100 × [Inhibition $(\%)_S$] − [Inhibition $(\%)_E$]/[Inhibition $(\%)_S$]; where [Inhibition $(\%)_S$] and [Inhibition $(\%)_E$] represent percent inhibition of erythrocytes AChE of saline controls and following treatment with eseroline, respectively. The values represent the means ± s.e.m. of 3 separate determinations performed in duplicate. Mean Inhibition $(\%)_S$ values were 82 and 96% for 100 μM physostigmine- and 10 μM DFP-pretreated erythrocytes, respectively.

	AChE inhibited by 100 μM Physostigmine reactivation (%)	AChE inhibited by 10 μM DFP reactivation (%)
Eseroline 100 μM	20 ± 5*	0 ± 0
Eseroline 300 μM	31 ± 5**	0 ± 1
Eseroline 1 mM	42 ± 6***	0 ± 0

*$P < 0·05$ as compared to saline controls; **$P < 0·02$ as compared to saline controls; ***$P < 0·01$ as compared to saline controls.

hydrolysis of the phosphorylated enzyme. On the other hand, an increased recovery of enzymic activity was evident in physostigmine-pretreated erythrocytes. This effect was, however, only manifest in the presence of relatively high concentrations of eseroline and probably reflected the ability of this drug to reverse the carbamylation of the enzyme to some extent.

In-vivo protection experiments

Eseroline salicylate (10 mg kg^{-1}) was administered s.c. to groups of mice 15 min before the injection of a 95% lethal dose of physostigmine (1 mg kg^{-1} i.p.) or of a 85% lethal dose of DFP (7 mg kg^{-1} s.c.). The animals did not receive any other treatment. The decrease in percent mortality among the poisoned animals pretreated with eseroline compared with those which had had saline alone was taken as an index of the protective activity of the drug. The results obtained are summarized in Table 3. It is seen that eseroline protected from death an average of 26 and 82% of the animals poisoned by physostigmine and DFP, respectively. The mean ± s.e.m. death latency in mice which did not survive physostigmine poisoning was 669 ± 30 and 356 ± 14 s depending whether or not they had been pretreated with eseroline, respectively ($P < 0.001$). In the case of DFP poisoning, eseroline pretreatment increased death latency from 516 ± 70 to 803 ± 29 s ($P < 0.01$).

The effect of the time-lapse between eseroline administration and poisoning was studied in separate experiments. It was seen that when eseroline was administered 5 min before the intoxicating agent, the percent lethality among mice was about the same as that observed at 15 min intervals, although the symptomatology of the intoxication in the surviving animals appeared milder. The percent protection afforded by the same dose of eseroline salicylate (10 mg kg^{-1} s.c.), on the other hand, was markedly lower (41% in 17 animals and 0% in 10 animals in the case of DFP and physostigmine, respectively) when the intoxicating agent was administered 30 min after the protector.

To test the possibility of this antidotal effect of eseroline being due to the opiate-like properties of the drug rather than to AChE protection, the effect of morphine hydrochloride (10 mg kg^{-1} s.c.) was evaluated against the poisoning by physostigmine under the same experimental conditions as used for eseroline. In a single experiment in 10 animals, morphine failed to prevent physostigmine lethality to any extent, but it did significantly ($P < 0.001$) increase death latency to 624 ± 54 s.

Table 3. Effect of prophylactic administration of eseroline salicylate (10 mg kg^{-1} s.c.) against physostigmine sulphate (1 mg kg^{-1} i.p.) and DFP (7 mg kg^{-1} s.c.) poisoning in mice. Eseroline and saline were administered 15 min before physostigmine or DFP. (n) = number of animals.

	(n)	Lethality (%)	Protection (%)
Saline + physostigmine	(40)	95 ± 2·9	
Eseroline + physostigmine	(60)	70 ± 8	26*
Saline + DFP	(20)	85 ± 5	
Eseroline + DFP	(20)	15 ± 5	82*

*$P < 0.02$ as compared to respective control groups.

DISCUSSION

These experiments provide evidence that the pretreatment of AChE preparations with eseroline prevents the inactivation of the enzyme by the organophosphate DFP and the carbamates physostigmine and neostigmine. The fact that in-vivo the prophylactic administration of eseroline protects mice against DFP and physostigmine poisoning corresponds to this in-vitro finding.

The protective action of eseroline on eel and erythrocyte AChE closely parallels the inhibitory pattern of the drug on these enzymic preparations. On the other hand, eseroline affords negligible or no protection at all to serum BuChE against the same inhibitors. Since eseroline is itself an extremely weak inhibitor of BuChE (Galli et al 1982), this finding confirms the close correlation existing between the inhibitory and the protective actions of eseroline on cholinesterases.

Eseroline is more effective in protecting AChE against DFP than against physostigmine and neostigmine, in that order. This is not due to a different degree of enzymic inactivation by these inhibitors, nor can such a difference be attributed to a structural analogy between eseroline and DFP molecules. It appears likely that the effectiveness of protection by eseroline resides largely in the time-course of the inhibitory action towards AChE of the agents used as inactivators. The rate of the inhibition progress of ChEs by organophosphates and carbamates is expressed by the value of their bimolecular reaction constant k_i, which is the ratio between the acylation constant, k_2, and the equilibrium dissociation constant, k_D (Main 1979). k_i for DFP, physostigmine and neostigmine on eel AChE were reported by Forsberg & Puu (1983) to be 1.58×10^2, 1.85×10^4 and 4.0×10^4 $M^{-1} s^{-1}$, respectively, i.e. the effec-

tiveness of eseroline protective action is inversely correlated to the k_i values of the anti-ChE agents used in our study as irreversible inhibitors. This reasoning confirms a previous observation by Depierre & Martin (1958).

The efficacy of eseroline treatment is much lower when it is added to the enzyme after the inhibitor and becomes progressively lower as the time lapse between the addition of the two drugs increases. Accordingly, eseroline does not reactivate the AChE which has been previously inhibited by DFP. However, high concentrations of the drug partially restore activity in the physostigmine-pretreated enzyme. This suggests that eseroline, while completely ineffective on the phosphorylated enzyme, is able to accelerate to a certain extent the regeneration of the free active enzyme from its carbamoylated derivative.

As we have pointed out above, the prophylactic administration of eseroline to mice protected significant percentages of the animals against poisoning by highly lethal doses of DFP and physostigmine. This result appears particularly noteworthy in that it was achieved in the absence of any supporting therapy with cholinolytics or reactivators (Berry & Davies 1970; Shoene et al 1976; Gordon et al 1978; Dirnhuber et al 1979). The antidotal properties of eseroline appear to be directly linked to its protective action on AChE, mainly for two reasons: in the first place, eseroline is more effective against DFP than against physostigmine, as happens in-vitro directly on AChE preparations; secondly, the protective action of eseroline does not seem to be due to the opiate-like properties of the drug, as in the same experiments morphine proved to be ineffective against physostigmine lethal effects.

The prophylactic action of eseroline takes place in mice at approximately 6–7 times lower doses than those responsible for the first mild symptoms, namely salivation and lacrimation, of cholinergic activation (unpublished observation). In this respect, therefore, eseroline compares favourably with carbamate protectors, which, like physostigmine and pyridostigmine, have been demonstrated to be effective against organophosphates at only slightly lower doses than those inducing cholinomimetic effects (Koster 1946; Berry & Davies 1970; Gordon et al 1978). On the other hand, the protective effect of eseroline is rather short-lasting, the maximum being reached 5–15 min after administration and rapidly declining afterwards.

Previous studies by Bergman & Shimoni (1952), Depierre & Martin (1958), Lüllmann et al (1967) and Shoene et al (1976) have shown that simple quaternary ammonium inhibitors of AChE may also protect this enzyme against irreversible anti-AChE agents. Unlike these compounds which would act prevalently in the periphery, eseroline readily crosses the blood-brain barrier and is expected to exert its protective action also on central AChE. Moreover, it is well known that ChE inhibitors which carry a quaternary nitrogen are particularly effective in potentiating skeletal muscular responses (Taylor 1980), and may cause involuntary muscular twitchings and fasciculations, even at relatively low concentrations. By contrast, in mice eseroline does not give rise to any symptomatology of muscular activation even at doses 10 times higher than those effective in the protection against anticholinesterases.

To sum up, eseroline protects AChE against inactivation by carbamates and DFP in-vitro and in-vivo, while it is practically ineffective on BuChE. The protection afforded by eseroline develops instantaneously in the presence of the enzyme and is particularly effective when DFP is used as an inactivator. These properties, together with the fact that this drug is practically devoid of cholinomimetic action, suggest that eseroline might be useful in the prevention of poisoning by organophosphate antiacetylcholinesterases.

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