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Prevention of physostigmine-induced lethality by the opioid analgesic meptazinol in the mouse

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1 The prophylactic action of meptazinol against physostigmine- and neostigmine-induced lethality was evaluated in mice. Meptazinol proved to be effective against physostigmine (1 mg kg^{-1} i.p.), but not against neostigmine (0.5 mg kg^{-1} i.p.).

2 The antagonism by meptazinol of physostigmine-induced poisoning was maximal when the drug was administered 15 min before physostigmine. Under these conditions the ED_{50} (95% confidence limits) of meptazinol was 24 (22.0–26.1) mg kg^{-1} s.c.. A 30 mg kg^{-1} dose of the drug prevented lethality in 89% of the animals.

3 The action of meptazinol was not antagonized by naloxone hydrochloride (2 mg kg^{-1} i.p.), injected 10 min before meptazinol.

4 Pretreatment of mice with 30 mg kg^{-1} meptazinol 15 min before physostigmine (1 mg kg^{-1}) poisoning increased brain acetylcholinesterase (AChE) activity on average, from 8 to 31% of control values.

5 The protection of cholinesterases against physostigmine- and neostigmine-induced inactivation was demonstrated *in vitro* directly on purified preparations of the enzymes using a dilution method. The ED_{50} values (95% confidence limits) for the protective effect of meptazinol of electric eel AChE against 1 and 3 μM physostigmine and 1 μM neostigmine were 2.6 (1.4–4.9), 9.5 (5–18) and 3 (1.6–5.7) μM, respectively, while for protection of horse serum butyrylcholinesterase (BuChE) against the same inhibitors, the ED_{50} values were 12 (5.4–26.4), 42 (27–65.1) and 8 (3.6–17.6) μM, respectively.

6 It is suggested that prevention of physostigmine-induced lethality by meptazinol is a consequence of its protective action on AChE in the central nervous system.

Introduction

Meptazinol (m-[1-methyl-3-ethyl-hexahydro-1H-azepin-3-yl]-phenol hydrochloride) is an opioid analgesic which is effective against moderate and severe pain of varying aetiologies (see Stephens *et al.*, 1978; Lancet Editorial, 1983; Holmes & Ward, 1985, for reviews). Interest in this drug also arises from reports that meptazinol's action does not wholly depend on its interaction with opioid receptors, a component of cholinergic activation being present in its pharmacological profile (Bill *et al.*, 1983; Wali, 1986). In fact, the response to meptazinol in various tests for antinociception in mice and rats is antagonized to different extents by the antimuscarinic agent scopolamine (Bill *et al.*, 1983). Furthermore when meptazinol is given in high doses it induces symptoms characteristic of cholinergic activation, which are reversed by scopolamine (Lancet Editorial, 1983). We recently demonstrated that meptazinol is endowed with remarkable inhibitory activity on cholinesterases from various sources and hypothesized that its cholinomimetic activity is the consequence of cholinesterase inhibition (Galli, 1985). This point has since been investigated by Strahan *et al.* (1985) and Ennis *et al.* (1986).

In a study undertaken to assess the pharmacological profile of meptazinol we found that it protected mice from the lethal effects induced by the anticholinesterase agent physostigmine. Since this finding was not apparently in line with the well-established cholinomimetic character of the drug, we thought this point worth examining more closely. This paper describes the results of our investigation.

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Methods

In vivo *experiments*

Groups of randomized male Swiss mice (25–28 g) were treated subcutaneously with meptazinol hydrochloride, or with saline, before the injection of lethal doses of physostigmine sulphate or neostigmine bromide. Lethality was assessed 90 min later, although the animals were under observation for an additional 24 h.

Brain acetylcholinesterase (AChE) activity in poisoned mice was measured by the photometric method of Ellman *et al.* (1961). Briefly, the animals were killed by decapitation 10 min after physostigmine (1 mg kg^{-1} i.p.) or saline (controls) administration. Brains were rapidly removed and homogenized in a glass-Teflon homogenizer (0.13–0.18 mm clearance) with 5 vol of ice-cold 0.05 M sodium phosphate buffer, pH 7.2; then 20 µl aliquots of the homogenates were mixed with 2.88 ml of 0.26 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in phosphate buffer directly in the spectrophotometer (Perkin-Elmer 552 S) cuvette and the reaction started by the addition of 100 µl of a 15 mM solution of acetylthiocholine iodide (ATCh) in buffer. The variations in optical absorbance (412 nm, 25°C) were measured for 1 min. The procedure was accomplished in less than 10 min. The preparation was found to contain almost exclusively AChE.

In vitro *experiments*

Measurement of cholinesterase inhibition Fifty µl aliquots (0.125–0.25 u) of purified AChE from *Electrophorus electricus* (Boehringer Mannheim Gmbh, 1000 u mg^{-1}) or butyrylcholinesterase (BuChE) from horse serum (Sigma Chemical Co. 13.3 u mg^{-1} protein) were made up to 2.85 ml with 0.25 mM DTNB in 0.05 M sodium phosphate buffer, pH 7.2, and preincubated at 25°C for 15 min with 6–7 graduated concentrations of meptazinol or buffer before being assayed for enzymic activity as described earlier, in the presence of 0.5 mM ATCh or 1 mM butyrylthiocholine iodide (BuTCh), respectively, as substrates. All values refer to final concentrations in the activity assay.

Measurement of the protective effect of meptazinol on cholinesterases Appropriate fixed amounts of test inhibitors, or buffer alone, were added to aliquots of eel AChE or horse serum BuChE previously mixed with graduated concentrations of meptazinol or buffer and incubated at 25°C for 15 min. Then 20 µl fractions of the incubates, corresponding to 0.4–0.6 u of enzymic activity were rapidly diluted 500 times with 0.25 mM DTNB and immediately assayed for AChE or BuChE activity in the presence of 0.5 mM ATCh or 1 mM BuTCh, respectively. The dilution of the samples and the calculation of reaction velocity were accomplished in less than 1 min. In the experiments designed to evaluate the effect of the addition times of meptazinol and physostigmine on AChE protection, the sequence of the addition of the two drugs was varied. However, in this case the 15 min incubation was also started from the moment of physostigmine addition. The drug concentration values in these experiments refer to final concentrations in the incubation mixtures, before dilution of the samples, while DTNB and substrate concentrations are those present in the activity assay.

Calculation of results

The % prevention of lethality was calculated from the expression:

$$\text{Lethality prevention (\%)} = \frac{[\text{Lethality (\%)}_P] - [\text{Lethality (\%)}_{M+P}]}{[\text{Lethality (\%)}_P]} \times 100$$

where [Lethality $(\%)_P$] and [Lethality $(\%)_{M+P}$] represent the % lethality following poison alone or meptazinol plus poison, respectively. The same equation was used to calculate cholinesterase protection *in vitro*: in this case enzyme inhibition was substituted for lethality in the expression.

Drugs

DTNB, ATCh and BuTCh were purchased from Boehringer Mannheim Gmbh. Physostigmine sulphate, neostigmine bromide and naloxone hydrochloride from Sigma Chemical Co. Ltd. Meptazinol hydrochloride was from Wyeth and levorphanol tartrate was a gift from Dr H. Gutman, F. Hoffmann-La Roche & Co., Basel. All drugs were administered in saline (0.9% w/v NaCl solution).

Results

Prevention of physostigmine-induced lethality

The results presented in Table 1 show the prophylactic effects of increasing doses of meptazinol against physostigmine-induced lethality in mice. Meptazinol was administered subcutaneously to randomized groups of mice 15 min before the injection of a 92% lethal dose of physostigmine sulphate (1 mg kg^{-1} i.p.). The decrease in % mortality among the poisoned animals pretreated with meptazinol compared with those which had received saline alone was taken as an index of the prophylactic activity of the drug. The effect was clearly dose-dependent and its ED_{50} (calculated accor-

Table 1 Prevention of physostigmine-induced lethality by increasing doses of meptazinol: lack of antagonism by naloxone

Pretreatment	*Drug dose* ($mg\,kg^{-1}$)	n	*Lethality* (%)	*Prevention of lethality* (%)	*Survival time* (min)
Saline	vol	51	92.1	0	6.7 ± 0.6
Meptazinol	20	30	66.6	27.7	$11.7 \pm 0.8^{***}$
Meptazinol	25	30	43.3	53.3	$13.2 \pm 0.9^{***}$, †
Meptazinol	30	40	10.0	89.1	$14.2 \pm 2.0^{***}$, †
Naloxone + meptazinol	2 30	15	6.6	92.8	13.5‡

Meptazinol hydrochloride, or saline, were injected s.c. 15 min before physostigmine sulphate ($1\,mg\,kg^{-1}$ i.p.). Naloxone hydrochloride was administered intraperitoneally 10 min before meptazinol. Survival time values are means ± s.e.mean. n = number of animals.
$^{***}P < 0.001$ vs saline, unpaired t test.
†Not significantly different from previous data point.
‡Only one animal in this group died.

ding to Litchfield & Wilcoxon, 1949; 95% confidence limits) was 24 (22.0–26.1) $mg\,kg^{-1}$. A dose of $30\,mg\,kg^{-1}$ of the opioid was effective in protecting 89.1% of the animals. The administration of the opioid antagonist naloxone ($2\,mg\,kg^{-1}$ i.p.) did not affect the prophylactic action of meptazinol. The data in Table 1 show that meptazinol pretreatment also increased considerably latency to death in mice which did not survive physostigmine poisoning. In surviving animals, however, it failed to improve substantially the symptoms of intoxication. Their behaviour normalized within 4 h. In our experiments physostigmine sulphate LD_{50}, calculated by probit analysis and based on the results obtained with 4 different doses of the drug administered intraperitoneally to 81 mice, was $0.73\,mg\,kg^{-1}$. When mice were pretreated with $30\,mg\,kg^{-1}$ meptazinol, physostigmine LD_{50}, calculated on 67 animals, was found to be $1.42\,mg\,kg^{-1}$. The ratio for the protective effect of meptazinol was, therefore, 1.87.

In a separate experiment the prophylactic action of meptazinol was also tested against neostigmine. The death rate in 20 mice poisoned with neostigmine bromide ($0.5\,mg\,kg^{-1}$ i.p.) 15 min after pretreatment with $30\,mg\,kg^{-1}$ meptazinol was 80%, whereas that in an equivalent number of saline-pretreated animals was 75%. Meptazinol, therefore, failed to prevent neostigmine-induced death to any extent.

To assess the effect of the time between meptazinol administration and physostigmine poisoning on the effectiveness of meptazinol's protective action, groups of mice were pretreated with $30\,mg\,kg^{-1}$ meptazinol, or saline alone, at different times (between 5 s–60 min) before the injection of $1\,mg\,kg^{-1}$ physostigmine sulphate. As shown in Table 2, an interval of 15 min between meptazinol and physostigmine afforded the

Table 2 Effect of time of administration of meptazinol and physostigmine on the prevention of lethality

Interval between meptazinol and physostigmine	n	*Prevention of lethality* (%)
5 s	20	15.7
5 min	20	68.4
15 min	40	89.1
30 min	20	27.7
60 min	20	11.1

Groups of mice were poisoned with physostigmine sulphate ($1\,mg\,kg^{-1}$) at intervals varying between 5 s and 60 min after the injection of meptazinol hydrochloride ($30\,mg\,kg^{-1}$ s.c.). The results were calculated as described in Methods using as controls an equivalent number of animals in which saline was substituted for meptazinol.
n = number of animals.

highest degree of lethality prevention. On the other hand, the prophylactic effect of meptazinol was very weak when the drug was injected either immediately before or 1 h before physostigmine.

Effect of meptazinol on brain acetylcholinesterase activity

The data in Table 3 show the effect of meptazinol pretreatment on brain AChE activity in mice poisoned with physostigmine. AChE assays were started 10 min after physostigmine administration and were carried out rapidly to minimize spontaneous enzymic activity recovery. Among the animals which had been treated with saline and physostigmine, only 2 out of 15 were still alive at the moment of death for brain removal, whereas all those pretreated with meptazinol were still alive at that time. The results in Table 3 show that in the poisoned unprotected animals only 8% of control AChE was still active. This value was increased to 31% in mice which had been pretreated with 30 mg kg^{-1} meptazinol. This increase is highly significant ($P < 0.001$) and represents a 25% protection of the enzyme. Meptazinol itself contributed to a small extent (5%) to AChE inhibition. Mean AChE activity in the two unprotected mice which were still alive 10 min after physostigmine administration did not significantly differ from that of the animals in the same group which had died: 9.2% versus 7.9%, respectively.

Protective effect of meptazinol on cholinesterases in vitro

Meptazinol has been shown to be a competitive inhibitor of AChE and BuChE (Galli, 1985). To evaluate its protective action on these enzymes, meptazinol had to be removed as completely as possible from the incubation medium before measurement of enzymic activity. In our experiments this was achieved by rapidly diluting, 500 times, the enzyme preparations which had been pre-incubated with meptazinol and inactivators before assaying residual cholinesterase activity. It was assumed that inhibition by physostigmine, like that by carbamate inhibitors in general, would not be significantly affected by the operation owing to the long-lasting character of the inhibitory effects of these drugs (Wilson *et al.*, 1960; Main & Hastings, 1966), while that of meptazinol should be greatly reduced or altogether abolished by extensive dilution, on account of its rapid reversibility (Galli, 1985).

Figure 1 shows the protective effects on electric eel AChE of increasing concentrations of meptazinol against fixed, highly inhibitory amounts of physostigmine and neostigmine. To enable a direct comparison with the inhibitory effect of the drug to be made, the inhibition curve of meptazinol on this enzyme has also been included in the same figure. The data show that meptazinol protected eel AChE against inactivation by both physostigmine and neostigmine. This action was dose-dependent and showed a pattern of progress which closely resembled that for inhibition. Meptazinol was about equally effective against 1 μM physostigmine and 1 μM neostigmine. Protection was significantly weaker against 3 μM physostigmine. The ED_{50} values (95% confidence limits) for such an action were 2.6 (1.4–4.9), 3.0 (1.6–5.7) and 9.5 (5–18) μM against 1 μM physostigmine, 1 μM neostigmine and 3 μM physostigmine, respectively. Under the same experimental conditions, meptazinol IC_{50} was 0.4 (0.2–0.8) μM. In the absence of meptazinol 1 μM physostigmine, 1 μM neostigmine and 3 μM physostigmine inhibited AChE by, on average, 96, 96 and 96.8%, respectively. The mean inhibition caused by 10^{-5} and 3×10^{-5} M meptazinol was on average 3 and 12%, respectively. This inhibition was not taken into account in the calculation of the results.

The results obtained on horse serum BuChE are shown in Figure 2. Meptazinol ED_{50} values for the

Table 3 Effect of meptazinol on brain acetylcholinesterase (AChE) activity in mice poisoned with physostigmine

Pretreatment	*Treatment*	n	*Brain AChE activity* $\mu mol\,g^{-1}\,min^{-1}$	*% of control*
Saline	Saline	10	7.9 ± 0.44	100
Meptazinol	Saline	5	7.5 ± 0.40†	95
Saline	Physostigmine	15	0.6 ± 0.05	8
Meptazinol	Physostigmine	10	2.4 ± 0.11***	31

Pretreatment of mice with saline or meptazinol (30 mg kg^{-1} s.c.) was carried out 15 min before i.p. treatment with saline or physostigmine sulphate (1 mg kg^{-1}): brains were removed for AChE assay 10 min later.
†Not significantly different (unpaired *t* test) from saline pretreated mice.
***$P < 0.001$ vs saline pretreated mice.

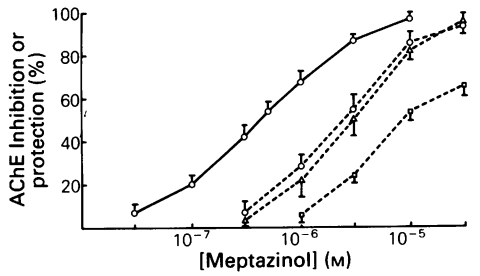

Figure 1 Inhibition of electric eel acetylcholinesterase (AChE) by meptazinol and its protection by the same drug against physostigmine and neostigmine. AChE inhibition (O——O); AChE protection against 1 μM (O- - -O) and 3 μM (□- - -□) physostigmine and 1 μM (Δ- - -Δ) neostigmine. Inhibition and protection values are expressed as percentages of uninhibited AChE activity of their respective controls. The points represent the mean of 3–4 separate experiments performed in duplicate and vertical lines show s.e.mean.

protection of this enzyme were 12 (5.4–26.4), 8 (3.6–17.6) and 42 (27–65.1) μM against 1 μM physostigmine, 1 μM neostigmine and 3 μM physostigmine, respectively. Meptazinol IC_{50} on horse serum BuChE was 3.6 (1.8–7.2) μM. The mean % inhibition of the enzyme by 1 μM physostigmine, 1 μM neostigmine and 3 μM physostigmine in the absence of protection by meptazinol was 97, 86.3 and 98.5%, respectively. In this system therefore both inhibition and protection by meptazinol developed at higher concentrations of the drug than those necessary for eel AChE. Meptazinol

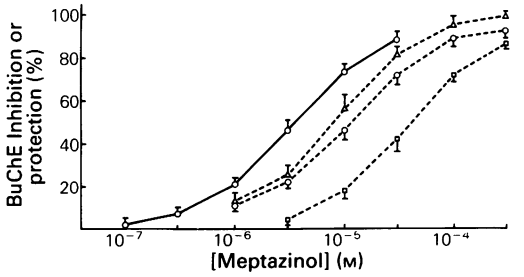

Figure 2 Inhibition of horse serum butyrylcholinesterase (BuChE) by meptazinol and its protection by the same drug against physostigmine and neostigmine. BuChE inhibition (O——O); BuChE protection against 1 μM (O- - -O) and 3 μM (□- - -□) physostigmine and 1 μM (Δ- - -Δ) neostigmine. Inhibition and protection values are expressed as percentages of uninhibited BuChE activity of their respective controls. The points represent the mean of 3–4 separate experiments performed in duplicate and vertical lines show s.e.mean.

was seen to be slightly more effective against neostigmine than against physostigmine. The inhibition caused by 10^{-4} and 3×10^{-4} M meptazinol alone was 3.4 and 13%, respectively.

In the experiments described so far meptazinol was added to the enzyme immediately before (about 3 s) physostigmine or neostigmine. In a series of separate experiments, the effect of the addition times of meptazinol and physostigmine on the protection of eel AChE was evaluated by adding meptazinol at different times after physostigmine. The results of these experiments are shown in Table 4. It can be seen that AChE protection by meptazinol is markedly diminished when the drug is added to the enzyme after physostigmine. In the samples in which meptazinol was added 15 min after physostigmine, that is immediately before dilution and activity assay, the protection was almost nil.

Discussion

The present results provide evidence that the opioid analgesic meptazinol is effective in preventing physostigmine-induced lethality in mice. This action developed at drug doses 2–5 times higher than those found to be analgesic in this animal (Bill *et al.*, 1983; Spiegel & Pasternak, 1984) and depended on the relative administration times of meptazinol and physostigmine, being maximal when meptazinol preceded physostigmine by 15 min.

Physostigmine-induced lethality is considered to be a specific expression of central cholinergic overstimulation. In fact, it has been reported that, while tertiary anticholinomimetic agents antagonize this action of physostigmine, quaternary (peripherally acting) anticholinomimetic drugs do not (Maayani *et al.*, 1977; Niemegeers *et al.*, 1982). In line with these findings, the antagonism of acute physostigmine poisoning in mice has been used by Nose & Kojima (1970) as a test for the screening of drugs for Parkinson's disease, some of the symptoms of which are consistent with an overactive central cholinergic system (Duvoisin, 1967; Lloyd, 1978). In the case of meptazinol, however, the prevention of physostigmine-induced lethality cannot be attributed to possible anticholinergic properties of the drug, since, on the contrary, meptazinol causes cholinergic stimulation (Stephens *et al.*, 1978; Bill *et al.*, 1983; Lancet Editorial, 1983; Wali, 1986). In addition, our experiments showed that meptazinol, unlike anticholinomimetic drugs (Berry & Davies, 1970; Taylor, 1985), exerts only a preventive action, being ineffective when poisoning is already in progress. The action of meptazinol does not appear to be mediated by an opioid receptor(s) either, since it was not antagonized by a dose of naloxone which has been shown to affect

Table 4 Effect of time between addition of meptazinol and physostigmine on the protection of electric eel acetylcholinesterase (AChE)

First addition	*Time between additions*	*Second addition*	*AChE protection*
Meptazinol	3 s	Physostigmine	84 ± 5
Physostigmine	3 s	Meptazinol	60 ± 5
Physostigmine	1 min	Meptazinol	32 ± 4
Physostigmine	2 min	Meptazinol	21 ± 3
Physostigmine	5 min	Meptazinol	15 ± 2
Physostigmine	15 min	Meptazinol	4 ± 2

Meptazinol (10 μM) and physostigmine (1 μM) were added to aliquots of electric eel AChE according to the indicated time sequence. The mixtures were then incubated at 25°C for 15 min, diluted 500 times with 0.25 mM DTNB and assayed for enzymic activity in the presence of 0.5 mM acetylthiocholine iodide. Values are the means ± s.e.mean of 3 separate experiments.

markedly the analgesic effects of the drug (Bill *et al.*, 1983). This finding is in agreement with previous observations that morphine and other opioids do not prevent physostigmine-induced lethality in mice and rats (Niemegeers *et al.*, 1982; Galli *et al.*, 1985). During this study we also tested the potent opioid levorphanol (15 mg kg^{-1} s.c.) and found that it was completely ineffective against physostigmine (unpublished observation).

It appears more likely that meptazinol exerts its prophylactic action by protecting cholinesterases. In fact, brain AChE activity in meptazinol-pretreated mice, was markedly higher than that in the animals which had received physostigmine alone (31% versus 8% of control activity). Although the minimum amount of AChE in brain compatible with life has not been clearly ascertained, to our knowledge, the increase in enzymic activity brought about by meptazinol appears to be high enough to explain its antidotal effects (DuBois *et al.*, 1949). Such a mechanism of action is also supported by our *in vitro* experiments. Meptazinol was found to protect effectively both AChE and BuChE against inhibition by physostigmine and neostigmine. The protective action of meptazinol closely paralleled the inhibitory patterns of the drug on the enzymes, thus showing that the two effects are strictly correlated. However, the concentrations of meptazinol protecting AChE were 6–20 times higher than inhibitory concentrations, suggesting that substantial inhibition of AChE is likely to occur *in vivo* at analgesic doses of the drug. We have previously demonstrated that the inhibitory action of meptazinol on AChE is instantaneous and reversible (Galli, 1985). By contrast, it is well known that cholinesterase inhibition by physostigmine and carbamates in general proceeds gradually through a time-dependent carbamylation step (Wilson *et al.*, 1960) and the regeneration of free active enzyme is a rather slow process (Main & Hastings, 1966). It can be hypothesized that protection of cholinesterases by meptazinol takes place on the formation of the Michaelis-Menten complex between inhibitor and enzyme. Meptazinol competes with physostigmine for the active site of the enzyme and thus prevents the formation of its stable carbamylated intermediate. This would cause a marked loss in the inhibitory potency of physostigmine and would considerably shorten its duration of action. The finding that AChE protection by meptazinol is markedly diminished when the drug is added to the enzyme after physostigmine is in agreement with this hypothesis. The K_D for the interaction between meptazinol and eel AChE is 8×10^{-8} M (Galli, 1985) while that between physostigmine and the same enzyme has been found to be 3.5×10^{-6} M (Brufani *et al.*, 1985). Therefore, in the competition between the two drugs for AChE, meptazinol appears to have a higher affinity for the enzyme.

It is well established that short-lasting cholinesterase inhibitors may protect these enzymes against inactivation by irreversible inhibitors (Koelle, 1946; Berry & Davies, 1970; Gordon *et al.*, 1978; Dirnhuber *et al.*, 1979; Harris & Stitcher, 1984; Singh *et al.*, 1986). In most cases, however, small doses of carbamate inhibitors, like physostigmine itself or pyridostigmine, were used to protect cholinesterases against organophosphorus derivatives. Our study has provided evidence that a reversible tertiary cholinesterase inhibitor, like meptazinol, can protect these enzymes even against physostigmine, indicating the inherently different character of the anti-cholinesterase activity of the two molecules. To our knowledge, the action of meptazinol is analogous only to that of the analgesic opioid, eseroline (Galli *et al.*, 1985). The protection afforded by meptazinol against physostigmine, however, appears to be more complete than that by

eseroline, and unlike the latter, meptazinol also protects BuChE.

Our results indicate that meptazinol is unable to prevent, to any extent, lethality induced in mice by the quaternary anticholinesterase agent neostigmine. However, meptazinol is highly effective in protecting both AChE and BuChE against this inhibitor *in vitro*. The lethality caused by neostigmine is considered to be peripherally-mediated since this drug unlike physostigmine, does not cross the blood-brain barrier appreciably (Rosecrans & Domino, 1974) nor does it affect brain AChE (Rosecrans & Domino, 1974; Maayani *et al.*, 1977). Besides, it has been reported that tertiary and quaternary anticholinomimetic drugs increase the neostigmine LD_{50} value in mice by the same factor (Maayani *et al.*, 1977). Our findings suggest, therefore, that meptazinol exerts its protective action mainly on AChE in the central nervous system.

In summary, this study has provided evidence that appropriate doses of the opioid analgesic meptazinol are highly effective in preventing physostigmine-induced lethality in mice. This property of meptazinol appears to be a consequence of its protection of central AChE against the long-lasting inhibition caused by physostigmine and is most likely due to meptazinol's ability to interact with cholinesterases with high affinity and in a reversible manner. This action of meptazinol is also of interest in view of its potential use in the prevention of poisoning by highly toxic organophosphorus compounds. This point is at present being investigated.

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References

BERRY, W.K. & DAVIES, D.R. (1970). The use of carbamates and atropine in the protection of animals against poisoning by 1,2,2-trimethylpropylmethylphosphonofluoridate. *Biochem. Pharmac.*, **19**, 927–934.

BILL, D.J., HARTLEY, J.E., STEPHENS, R.J. & THOMPSON, A.M. (1983). The antinociceptive activity of meptazinol depends on both opiate and cholinergic mechanisms. *Br. J. Pharmac.*, **79**, 191–199.

BRUFANI, M., LIPPA, S., MARTA, M., ORADEI, A. & POMPONI, M. (1985). AChE inhibition by eserine. Rate constants of reaction. Part II. *It. J. Biochem.*, **34**, 328–340.

DIRNHUBER, P., FRENCH, M.C., GREEN, D.M., LEADBEATER, L. & STRATTON, J.A. (1979). The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J. Pharm. Pharmac.*, **31**, 295–299.

DUBOIS, K.P., DOULL, J., SALERNO, P.R. & COON, J.M. (1949). Studies on the toxicity and mechanism of action of p-nitrophenyl diethyl thionophosphate (parathion). *J. Pharmac. exp. Ther.*, **95**, 79–91.

DUVOISIN, R.C. (1967). Cholinergic-anticholinergic antagonism in parkinsonism. *Arch. Neurol.*, **17**, 124–136.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. JR. & FEATHERSTONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

ENNIS, C., HAROUN, F. & LATTIMER, N. (1986). Can the effects of meptazinol on the guinea-pig isolated ileum be explained by inhibition on acetylcholinesterase? *J. Pharm. Pharmac.*, **38**, 24–27.

GALLI, A. (1985). Inhibition of cholinesterases by the opioid analgesic meptazinol. *Biochem. Pharmac.*, **34**, 1579–1581.

GALLI, A., MALMBERG, AIELLO, P., RENZI, G. & BARTOLINI, A. (1985). In-vitro and in-vivo protection of acetylcholinesterase by eseroline against inactivation by diisopropyl fluorophosphate and carbamates. *J. Pharm. Pharmac.*, **37**, 42–48.

GORDON, J.J., LEADBEATER, L. & MAIDMENT, M.P. (1978). The protection of animals against organophosphate poisoning by pretreatment with a carbamate. *Tox. Appl. Pharmac.*, **43**, 207–216.

HARRIS, L. & STITCHER, D. (1984). Protection against diisopropylfluorophosphate intoxication by pyridostigmine and physostigmine in combination with atropine and mecamylamine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **327**, 64–69.

HOLMES, B. & WARD, A. (1985). Meptazinol. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. *Drugs*, **30**, 285–312.

KOELLE, G.B. (1946). Protection of cholinesterase against irreversible inactivation by di-isopropyl fluorophosphate in vitro. *J. Pharmac. exp. Ther.*, **88**, 232–237.

LANCET EDITORIAL (1983). Meptazinol. *Lancet*, **ii**, 384–385.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, **96**, 99–113.

LLOYD, K.G. (1978). Neurochemical compensation in Parkinson's disease. In *Parkinson's Disease: Concepts and Prospects*. ed. Lakke, J.P., Korf, J. & Wesseling, H. pp. 61–72. Amsterdam: Excerpta Medica.

MAAYANI, S., EGOZI, Y., PINCHASI, I. & SOKOLOVSKI, M. (1977). Characterization of some effects induced by physostigmine in mice: in vivo and in vitro studies. *Biochem. Pharmac.*, **27**, 203–211.

MAIN, A.R. & HASTINGS, F.L. (1966). Carbamylation and binding constants for the inhibition of acetylcholinesterase by physostigmine. *Science*, **154**, 400–402.

NIEMEGEERS, C.J.E., AWOUTERS, F., LENAERTS, F.M., VERMEIRE, J. & JANSSEN, P.A.J. (1982). Prevention of physostigmine-induced lethality in rats. A pharmacological analysis. *Archs. int. Pharmacodyn.*, **259**, 153–165.

NOSE, T. & KOJIMA, M. (1970). A simple screening method for antiparkinsonian drugs in mice. *Eur. J. Pharmac.*, **10**, 83–86.

ROSECRANS, J.A. & DOMINO, E.F. (1974). Comparative effects of physostigmine and neostigmine on acquisition and performance of a conditioned avoidance behavior in the rat. *Pharmac. Biochem. & Behav.*, **2**, 67–72.

SINGH, A.K., ZELEZNIKAR, R.J. Jr. & DREWES, L.R. (1986). Protection from quinidine or physostigmine against in vitro inhibition by sarin of acetylcholinesterase activity. *Life Sci.*, **38**, 165–172.

SPIEGEL, K. & PASTERNAK, G.W. (1984). Meptazinol: a novel mu-1 selective opioid analgesic. *J. Pharmac. exp. Ther.*, **228**, 414–419.

STEPHENS, R.J., WATERFALL, J.F. & FRANKLIN, R.A. (1978). A review of the biological properties and metabolic disposition of the new analgesic agent, meptazinol. *Gen. Pharmac.*, **9**, 73–78.

STRAHAN, S.K., PLEUVRY, B.J. & MODLA, C.Y. (1985). Effect of meptazinol on neuromuscular transmission in the isolated rat phrenic nerve-diaphragm preparation. *Br. J. Anaesth.*, **57**, 1095–1099.

TAYLOR, P. (1985). Anticholinesterase agents. In *The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Goodman, L.S., Rall, T.W. & Murad, F. pp. 110–129. New York: Macmillan Publ. Co.

WALI, F.A. (1986). On the cholinergic component of action of meptazinol. *Acta physiol. pharmac. latinoam.*, **36**, 77–87.

WILSON, I.B., HATCH, M.A. & GINSBURG, S. (1960). Carbamylation of acetylcholinesterase. *J. biol. Chem.*, **235**, 2312–2315.

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