[15], despite the antinicotinic properties that have been ascribed to it in other systems [1, 3, 4, 7, 8]. Similarly, the effects of aprophen on brain benzodiazepine receptors and on AChE are of no therapeutic significance.

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Inhibition of cholinesterases by the opioid analgesic meptazinol

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Meptazinol (*m*-[1-methyl-3-ethyl-hexahydro-1H-azepin-3-yl]-phenol hydrochloride (Fig. 1), is a new agonist/antagonist opioid analgesic which is effective against moderate and severe pain of varying aetiologies [1–3] and in some types of shock [4–6]. The interest in this opioid also arises from the reports that it is practically devoid of psychomimemtic side-effects [1, 7], that it affects respiration only minimally [3, 8], and that it has an extremely low dependence potential [9].

Recent findings suggest that meptazinol action does not wholly depend on the interaction with opiate receptors, a component of cholinergic activation being present in the pharmacological profile of the drug. In fact: in contrast to opioid drugs in general, meptazinol potentiates the electrically-induced twitch response of the guinea-pig isolated ileum [10]; the response to meptazinol in various tests for antinociception in mice and rats is antagonized to various extents by the antimuscarinic agent scopolamine [11]; when meptazinol is given in high doses it induces an evident symptomatology of cholinergic activation, which is reversed by scopolamine [12]. Accordingly, it has been proposed that some of the pharmacological properties of meptazinol, namely antinociception [11] and antipyresis [13], may be the result of a dual action of the drug at the opioid receptors and on cholinergic mechanisms.

In spite of the number of studies to which meptazinol has been subjected, no experimental data, to our knowledge, have as yet been reported to explain the mechanism of cholinergic activation by this drug. To acquire information on this important point, we have examined in the present investigation the action of meptazinol on cholinesterases (ChE) from various sources. This approach was motivated by the observation that opioid drugs possess varying degrees of anti-ChE activity [14–17]. If this property turned out to be particularly marked in meptazinol, it would provide a plausible explanation for the cholinomimetic actions of the drug, since it is well known that ChE inhibitors may indirectly induce cholinergic activation [18].

Materials and methods

Purified AChE (1000 units/mg) from *Electrophorus electricus*, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCh) and butyrylthiocholine iodide (BuTCh) were purchased from Boehringer Mannheim GmbH (F.R.G.). Human erythrocyte (RBC) AChE (1.25

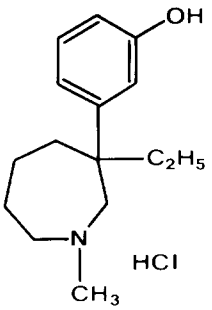

Fig. 1. Chemical structure of meptazinol.

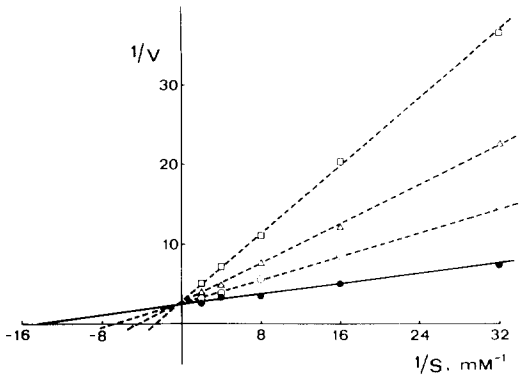

Fig. 2. Inhibition of electric eel AChE by meptazinol: Lineweaver–Burk plots. The assay conditions are described in Methods: ●—●, buffer; ○ - - - ○, 0.125 μM, △ - - - △, 0.25 μM; □ - - - □, 0.5 μM meptazinol; V = Δ absorbance/min; S = ATCh. The points of the graph are the means of four experiments performed in duplicate.

units/mg) and horse serum BuChE (13.3 units/mg protein) were from Sigma Chemical Co. (St. Louis, MO). Meptazinol was kindly donated by Dr. D. Green (Wyeth Laboratories, Maidenhead, Berks, U.K.). A 20% w/v homogenate of whole rat (300 g, Sprague–Dawley) brain in 0.05 M sodium phosphate buffer, pH 7.2, prepared according to Moss and Fahrney [19], was used as a source of brain ChE. The final dilution of brain tissue in the assay system was 1:1500. The preparation was found to contain almost exclusively AChE.

ChE activity was measured at 25° and pH 7.2 by the photometric method of Ellman *et al.* [20] using ATCh or BuTCh as substrates.

In the saturation experiments, designed to evaluate K_i values, 0.1 ml aliquots of 3 scalar concentrations of meptazinol were added directly in the spectrophotometer cuvette to 50 μl of the enzyme preparations (0.05–0.125 units) and to 2.75 ml of 0.27 mM DTNB in 0.05 M sodium phosphate buffer, pH 7.2. Immediately afterwards, 0.1 ml of 5 scalar concentrations of ATCh in the range 0.031–0.5 mM, or for horse serum BuChE, of 6 scalar concentrations of BuTCh in the range 0.031–1 mM, were added and rapidly mixed. The increase in optical absorbance at 412 nm was measured during a 30- or 60-sec (rat brain) interval by means of a Perkin-Elmer 552 S spectrophotometer, starting 15 sec after the addition of substrate. The temperature was maintained at 25° throughout the assays. All enzyme, substrate and meptazinol solutions were prepared in sodium phosphate buffer, pH 7.2. The concentration values refer to final concentrations present in the 3 ml sample used in the activity assay.

In order to evaluate the influence of the contact time between enzyme and inhibitor on ChE inhibition by meptazinol, 50 μl aliquots of eel AChE (0.125 units) were preincubated in 2.75 ml of DTNB with a fixed concentration of the drug for varying times (0–60 min) before the enzymic hydrolysis was started by adding 0.1 ml ATCh (0.5 mM). The increase in optical absorbance during a 30-sec period was measured.

The reversibility of the inhibitory action of meptazinol was evaluated according to the following procedure: two 50 μl portions of a solution containing electric eel AChE (1 unit/ml), preincubated in the presence of 3 μM meptazinol for 15 min at room temperature, were diluted to 2.9 ml, one with DTNB in 3 μM meptazinol and the other with DTNB in buffer. Both samples were then assayed for enzymic activity after the addition of 0.1 ml of ATCh (0.5 mM) at various times after dilution. The enzyme activity was assayed as the increase of absorbance during 30 sec.

Results and discussion

Meptazinol inhibited all the ChE preparations tested in a competitive, or prevalently competitive way. The corresponding K_i values computed from double-reciprocal plots are given in Table 1. Figure 2 shows, as an example, the plots obtained with electric eel AChE using three different concentrations of meptazinol; the graphs obtained with the enzymic preparations from rat brain, human RBC and horse serum were comparable. The results of Table 1 show that meptazinol was appreciably more potent on eel AChE ($K_i = 0.08 \pm 0.01$ μM) than on the other ChE. On the other hand, the potency of the drug on horse serum BuChE ($K_i = 0.98 \pm 0.15$ μM) did not differ markedly from that observed in rat brain ($K_i = 0.52 \pm 0.12$ μM) and human erythrocyte ($K_i = 0.92 \pm 0.28$ μM) AChE.

These results suggest that the cholinergic activation caused by meptazinol is a consequence of its anti-ChE action. In fact, it has been reported that cholinergic participation in the antinociceptive effects of meptazinol in rats and mice is evident at doses of the drug in the range 16–25 mg/kg s.c. [11]. Although it is difficult to generalize about the *in vivo* situation from the *in vitro* one, it seems very likely from our results and from previous meptazinol plasma kinetics data [22] that such doses of the drug are high enough markedly to inhibit ChE. The same kind of reasoning can be applied to the observation that cholinergic side-effects are evident when meptazinol is given in high doses as a premedication and during anaesthesia [12]. It appears likely that also in these situations the circulating drug attains high enough levels considerably to affect the ChE activity of the organism.

Our experiments showed that meptazinol action on ChE is time-independent. This was ascertained by preincubating aliquots of eel AChE (0.125 units) with a fixed concentration of meptazinol for periods of time varying from 0 to 60 min before adding ATCh (0.5 mM) to measure enzymic activity. It was seen that the initial AChE inhibition (51.3 ± 3% at 0.5 μM meptazinol) remained practically unmodified in all the samples tested, independently of preincubation duration. Since enzyme assays took 45 sec to perform, meptazinol inhibitory action was fully developed in less, and probably much less, than this period of time. Accordingly, when eel AChE preincubated with 3 μM meptazinol was rapidly diluted 60 times (final meptazinol concentrations equalling 0.05 μM) and immediately assayed for activity, AChE inhibition decreased from 88 ± 4% to 15 ± 2%. If time periods of as much as 30 min were allowed to elapse between dilution and assays, no further enzyme recovery increase was observed. In other words, meptazinol-treated AChE had regained on dilution its maximum theoretical activity within the 45 sec time period required for measuring the reaction velocity.

The time-course of the inhibitory action of meptazinol on ChE differs markedly from that of physostigmine and

Table 1. Inhibition constants (pH 7.2, 25°) of meptazinol towards ChE from various sources

Inhibition constant	Electric eel* (μM)	Human RBC* (μM)	Rat brain* (μM)	Horse serum† (μM)
K_i	0.08 ± 0.01	0.92 ± 0.28	0.52 ± 0.12	0.98 ± 0.15

K_i values were calculated from Lineweaver–Burk plots (see Fig. 2) by replotting the slopes of the regression lines against meptazinol concentrations: the intercepts on base line gave K_i [21]. Three different concentrations of meptazinol were used for each enzyme preparation. The conditions of the activity assay are reported in Methods. The figures are the means ± S.E.M. of four separate determinations performed in duplicate.

* ATCh (0.031–0.5 mM) as substrate.

† BuTCh (0.031–1 mM) as substrate.

of carbamate inhibitors in general. The action of meptazinol is time-independent and therefore instantaneously reversible on dilution. By contrast, inhibition of ChE by carbamates proceeds gradually [23] and the regeneration of free active enzyme after heavy dilution is a rather slow process [24]. In this respect, the action of meptazinol more closely resembles that of the ammonium phenols, like edrophonium, which inhibit ChE in a truly reversible manner [25, 26]. Meptazinol, however, does not carry the quaternary nitrogen which is known particularly to potentiate the neuromuscular effects of these agents [18]. These observations could explain the failure to detect the anti-ChE activity of the drug with the rat phrenic nerve diaphragm preparation [10].

To sum up, this study has provided evidence that the opioid analgesic meptazinol is endowed with remarkable inhibitory activity towards ChE from different sources, its K_i values for the enzymes tested ranging from 0.08 to 0.98 μM. In contrast to physostigmine and carbamate inhibitors in general, meptazinol anti-ChE action is truly reversible. These results appear consistent with the hypothesis that the cholinomimetic actions of meptazinol are consequence of ChE inhibition by this drug.

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