Biochemical Pharmacology, Vol. 34, No. 9, pp. 1577–1579, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

SHORT COMMUNICATIONS

Comparative effects of aprophen, atropine and benactyzine on central and peripheral cholinoceptors and on acetylcholinesterase

(*Received* 5 *March* 1984; *accepted* 31 *July* 1984)

Aprophen [the 2-(diethylamino)ethyl ester of α-methyl-α-phenylbenzeneacetic acid] is a drug with a number of distinct pharmacological actions [1–10]. These include its cholinolytic action as both a muscarinic and a nicotinic antagonist [1, 3, 4, 7, 8]. Either of these effects could contribute to the efficacy of aprophen in therapy for poisoning by organophosphate anticholinesterase agents [11–14]. Thus, the muscarinic antagonist atropine has long been the standard drug of this type for such therapy, while Su *et al.* [15] have proposed that the nicotinic antagonistic properties of bispyridinium oximes play an important part in their antidotal efficacy. Accordingly, it is important to quantitate the affinity of aprophen for muscarinic and nicotinic cholinoceptors. In a recent communication in this journal, Gordon *et al.* [16] reported on the ability of aprophen to compete with the muscarinic ligand [^{3}H]quinuclidinyl benzilate (QNB) for binding to N4TG1 neuroblastoma cells and NG108-15 neuroblastoma × glioma hybrid cells. The inhibition constants (K_i) for aprophen calculated from their results (4.3 and 0.7×10^{-6} M respectively) suggest that aprophen is a relatively weak antagonist at muscarinic receptors, since K_i for atropine for brain homogenates is of the order of 10^{-9} M [17]. Moreover, atropine was not included in the study so the relative potencies of atropine and aprophen in the above cell cultures are unknown.

We have compared the affinity of aprophen with that of atropine and benactyzine for muscarinic cholinoceptors in brain, ileum and heart of the guinea pig, and report below on the results. Benactyzine is structurally similar to aprophen and differs only in having a hydroxyl group rather than a methyl group on the α-carbon atom. Benactyzine is also an effective antidote to organophosphate anticholinesterase agents [18]. We have also evaluated the affinity of aprophen for rat brain nicotinic receptors and guinea pig brain benzodiazepine receptors and studied the interaction between aprophen and the enzyme acetylcholinesterase (AChE; EC 3.1.1.7). Benzodiazepine receptors were included in the study because the anticonvulsant diazepam has been shown to be an effective component of therapeutic mixtures [14].

Materials and methods

l-Quinuclidinyl[*phenyl*-4-^{3}H]benzilate (44 Ci/mmole), acetyl[*methyl*-^{3}H]choline chloride (86 Ci/mmole) and [*N-methyl*-^{3}H]flunitrazepam (72 Ci/mmole) were obtained from Amersham, Australia. Aprophen was a gift from the Chemical Defence Establishment, Porton, U.K. Other materials used were obtained commercially.

The decrease in force of contraction of the guinea pig atrium with acetylcholine chloride as the agonist at 37° was used to study the muscarinic receptor of this tissue. Aprophen, atropine and benactyzine were competitive antagonists in this system, and their apparent dissociation constants (K_i) were determined from a Schild plot of the data [19]. Direct binding assays were used for all other receptor systems. Muscarinic receptors of guinea pig brain and ileum were assayed with [^{3}H]QNB (44–74 pM; Refs. 20 and 21). Benzodiazepine receptors of guinea pig hippocampus were assayed with [^{3}H]flunitrazepam (0.7 nM) in Krebs phosphate buffer (pH 7.4) at 2° [17]. Nicotinic receptors of rat cerebral cortex were prepared and assayed in a Tris buffer with [^{3}H]acetylcholine (10 nM) as described by Schwartz *et al.* [22] except that GF/B filters were used instead of GF/C filters and Beckman scintillant HP was used instead of Liquiscint. It was verified that the HP scintillant extracted the tritiated material from the filter into solution with high efficiency (>85%; Ref. 23). For all ligands, inhibition of binding by the cholinergic drugs was analysed by a Hill plot. The Hill coefficient (n_H), and the dissociation constant of the drug (K_i) were calculated from the parameters of the Hill plot [17]. Values of the ligand-receptor dissociation constant (K_d) necessary for the calculation of K_i were determined in separate experiments under the same experimental conditions. These values of K_d have been published in the case of muscarinic and benzodiazepine receptors [17, 21]. Results for the nicotinic receptor are given below; in this case five concentrations of [^{3}H]acetylcholine (4–40 nM) were used, and the data were analysed as described in Ref. 24.

Enzyme studies with bovine erythrocyte AChE from Sigma (U.S.A.) were performed as described previously [24, 25] except that the buffers for dephosphorylation and ageing at 37° were 2.5 mM phosphate–150 mM NaCl and 2 mM phosphate–10 mM NaCl respectively.

Protein assays were performed using a modified Lowry method [26].

Results and discussion

The results of muscarinic receptor assays are given in Table 1. Atropine inhibited binding of [^{3}H]QNB to homogenates of guinea pig brain and ileum with a value of K_i in the nanomolar range. This value was significantly higher for the pons-medulla (1.02 nM) than for the striatum (0.56 nM) as reported previously [17], while it was higher still in the ileum (2.15 nM). Aprophen was also a potent inhibitor of [^{3}H]QNB binding and was approximately four times less effective than atropine (as indicated by the comparative K_i values) for both brain regions and the ileum. Benactyzine was found to be equipotent with aprophen in the striatum and ileum, but only half as effective in the pons-medulla (Table 1). In all cases, the value of the Hill coefficient n_H was statistically indistinguishable from 1.0. This indicates a lack of co-operativity in binding and is characteristic of muscarinic antagonists [27]. Comparable results to those above were obtained from the organ bath experiments with guinea pig atrium (Table 1). Thus, atropine exhibited a K_i value of 2.16 nM, and the corresponding values for aprophen and benactyzine were four to five times higher (i.e. lower affinity). The above results are consistent with those of Metcalfe [28], who found atropine and aprophen to inhibit binding of [^{3}H]QNB to whole porcine brain (minus cerebellum) with I_{50} values of 1.0 and 1.6 nM respectively. On the other hand, values of K_i for aprophen in guinea pig tissues (Table 1) are three orders of magnitude lower than those reported by Gordon *et al.* [16] for neuronal cell cultures.

Nicotinic receptors of rat brain (cerebral cortex) were assayed using [^{3}H]acetylcholine in the presence of atropine to block muscarinic receptors and diisopropylfluorophosphate (DFP) to inactivate AChE [22]. The value of K_d

Table 1. Apparent dissociation constants and Hill coefficients of antagonists of muscarinic cholinoceptors of guinea pig brain, ileum and atrium*

Drug	Tissue			
	Striatum†	Pons-medulla†	Ileum†	Atrium‡
		K_i (nM)		
Atropine	0.56 ± 0.05	1.02 ± 0.10	2.15 ± 0.15	2.16 ± 0.05
Aprophen	2.29 ± 0.32	3.75 ± 0.23	8.00 ± 1.68	10.30 ± 0.51
Benactyzine	3.42 ± 0.61	7.10 ± 0.45	9.35 ± 0.85	7.80 ± 0.22
		n_H		
Atropine	1.03 ± 0.06	1.06 ± 0.05	0.95 ± 0.05	
Aprophen	1.08 ± 0.03	1.26 ± 0.09	1.01 ± 0.04	
Benactyzine	0.96 ± 0.06	1.05 ± 0.02	1.08 ± 0.08	

* Data are quoted as mean ± S.E.M.
† [^{3}H]QNB binding assay; N = 4.
‡ Organ-bath experiment (decrease in force of contraction; acetylcholine as agonist); N = 5.

(ligand-receptor dissociation constant) was found to be 8.3 ± 1.5 nM (S.E.; N = 5), and B_{max} (calculated total concentration of receptor) was found to be 3.6 ± 0.3 pmoles/g tissue, or 66.8 ± 8.3 fmoles/mg protein (N = 5). The results are consistent with those of Schwartz *et al.* [22] who found K_d and B_{max} to be 12.3 nM and 4.6 pmoles/g tissue respectively. Schwartz *et al.* also reported that the omission of DFP led to higher nonspecific binding but lower specific binding than in its presence [22]. The same behaviour was observed in our experiments. Hill plots in the presence of aprophen were linear with a mean slope (n_H) of 1.00 ± 0.08 (S.E.; N = 4) i.e. no co-operativity was apparent. The mean value of K_i was found to be 0.19 ± 0.03 mM (N = 4). Because the muscarinic cholinoceptors of rat brain have very similar properties to those of guinea pig brain [17], we conclude that aprophen is five orders of magnitude weaker as a nicotinic antagonist than it is as a muscarinic antagonist. As a nicotinic antagonist, it is only two and one-half times as effective as atropine (K_i = 0.48 mM; Ref. 22). Further, Gordon *et al.* [16] found that aprophen (of unspecified concentration) has no discernible effect on the binding of the nicotinic ligand [^{3}H]tubocurarine to NG108-15 cells.

Aprophen was a weak inhibitor of the binding of [^{3}H] flunitrazepam to benzodiazepine receptors of guinea pig hippocampus. Values of n_H and K_i were found to be 1.03 ± 0.05 (N = 3) and 0.65 ± 0.02 mM (N = 3) respectively.

The interactions of aprophen with AChE were also studied. Aprophen was found to be a mixed competitive–uncompetitive inhibitor of the hydrolysis of acetylthiocholine by AChE. The competitive and uncompetitive dissociation constants were found to be 0.27 ± 0.12 mM (S.E.; N = 6) and 2.9 mM (range 0.9 to 4.6 mM) respectively. The figures are somewhat similar to those for benactyzine under the same conditions, 0.63 mM and 20.7 mM respectively [24]; both compounds are comparatively weak inhibitors. Aprophen at the relatively high concentration of 1 mM had negligible effects on the rates of ageing of isopropylmethylphosphonyl-AChE and dephosphorylation of diethylphosphoryl-AChE, but inhibited the rate of decarbamylation of dimethylcarbamyl-AChE by 32% (Table 2). Again, aprophen has similar effects to benactyzine which inhibited decarbamylation with a dissociation constant for the benactyzine–dimethylcarbamyl-AChE complex of 2.9 mM [24]. Decarbamylation occurs by hydrolysis, so it would appear that aprophen and benactyzine inhibit decarbamylation by hindering the accessibility of water to the esteratic site. We did not attempt to distinguish the two ways in which this could happen, i.e. binding to the "anionic" site in close proximity to the dimethylcarbamyl group, thereby shielding it from the nucleophile water, or binding to an allosteric site [29] with a consequent conformational change at the active site.

The results of the present study indicate that the therapeutic efficacy of aprophen with respect to organophosphate anticholinesterase agents is probably due to its antimuscarinic activity. Aprophen is unlikely to produce a direct antagonism of nicotinic receptors in brain *in vivo*

Table 2. Effect of aprophen on rate constants for reactivation and ageing of inhibited acetylcholinesterase*

	Rate constant k (hr^{-1})		
	Dephosphorylation	Decarbamylation	Ageing
Control	0.0147 ± 0.0010 (3)	0.344 ± 0.014 (4)	0.315 ± 0.009 (2)
1 mM Aprophen	0.0162 ± 0.0008 (2)	0.235 ± 0.013† (5)	0.288 ± 0.030 (2)

* Data are mean ± S.E.M., with the number of experiments in parentheses. Inhibited AChE = diethylphosphoryl-AChE (for dephosphorylation), dimethylcarbamyl-AChE (for decarbamylation), and isopropylmethylphosphonyl-AChE (for ageing).
† Significantly different from control, $P < 0.05$.

[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.

Acknowledgement—We are grateful to the Director of the Chemical Defence Establishment, Porton Down, U.K., for the gift of the sample of aprophen.

Materials Research Laboratories RAYMOND M. DAWSON*
Department of Defence SHIRLEY E. FREEMAN
Ascot Vale, Vic. 3032, Australia BRIAN M. PADDLE

REFERENCES

1. P. P. Denisenko, *Chem. Abstr.* **55**, 1933i (1961).
2. P. P. Denisenko, *Chem. Abstr.* **61**, 13771h (1964).
3. S. S. Liberman, *Chem. Abstr.* **53**, 12465e (1959).
4. Y. V. Burov and I. G. Kurochkin, *Chem. Abstr.* **79**, 13491 (1973).
5. M. V. Pan'shina, *Chem. Abstr.* **60**, 12555f (1964).
6. S. V. Anichkov and P. P. Denisenko, *Medna exp.* **1**, 255 (1959).
7. A. L. Mndzhoyan and V. M. Avakyan, *Chem. Abstr.* **63**, 7509c (1965).
8. E. E. Fomicheva, *Chem. Abstr.* **86**, 133513 (1977).
9. M. M. Rotmistrov, D. F. Pashkovskaya and S. S. Stavskaya, *Chem. Abstr.* **72**, 110203 (1970).
10. G. A. Buznikov, N. D. Zvezdina, N. V. Prokazova, B. N. Manukhin and L. D. Bergel'son, *Experientia* **31**, 902 (1975).

* Address all correspondence to: Dr. R. M. Dawson, Materials Research Laboratories, Department of Defence, P.O. Box 50, Ascot Vale, Vic. 3032, Australia.

11. N. K. Byalko, *Chem. Abstr.* **55**, 1924b (1961).
12. A. E. Didenko, *Chem. Abstr.* **58**, 2773e (1963).
13. V. B. Prozorovskii, *Chem. Abstr.* **70**, 10039 (1969).
14. G. D. D'Mello, in *Proc. Int. Symp. Protection against Chem. Warfare Agents*, FOA Report C40171, p. 89. National Defence Research Institute, Umea, Sweden (1983).
15. C-T. Su, C-P. Tang, C. Ma, Y-S. Shih, C-Y. Liu and M. T. Wu, *Fund. appl. Toxic.* **3**, 271 (1983).
16. R. K. Gordon, F. N. Padilla, E. Moore, B. P. Doctor and P. K. Chiang, *Biochem. Pharmac.* **32**, 2979 (1983).
17. R. M. Dawson and M. Poretski, *Neurochem. Int.* **5**, 369 (1983).
18. J. H. Wills, in *Handbuch der Experimentallen Pharmakologie* (Ed. G. B. Koelle), Vol. 15, p. 905. Springer, Berlin (1963).
19. R. J. Tallarida and R. B. Murray, *Manual of Pharmacologic Calculations with Computer Programs*, pp. 29–31. Springer, New York (1981).
20. R. M. Dawson and B. Jarrott, *Neurochem. Res.* **5**, 809 (1980).
21. R. M. Dawson and B. Jarrott, *Biochem. Pharmac.* **30**, 2365 (1981).
22. R. D. Schwartz, R. McGee and K. J. Kellar, *Molec. Pharmac.* **22**, 56 (1982).
23. R. M. Dawson, *Analyt. Biochem.* **139**, 493 (1984).
24. R. M. Dawson and M. P. Bladen, *Biochem. Pharmac.* **28**, 2211 (1979).
25. R. M. Dawson, *J. Neurochem.* **30**, 865 (1978).
26. E. F. Hartree, *Analyt. Biochem.* **48**, 422 (1972).
27. E. C. Hulme, N. J. M. Birdsall, A. S. V. Burgen and P. Mehta, *Molec. Pharmac.* **14**, 737 (1978).
28. R. F. Metcalfe, *Biochem. Pharmac.* **30**, 209 (1981).
29. R. M. Dawson, M. Poretski and C. M. Upsher, *Neurochem. Int.* **3**, 405 (1981).

Biochemical Pharmacology, Vol. 34, No. 9, pp. 1579–1581, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Inhibition of cholinesterases by the opioid analgesic meptazinol

(*Received* 29 *June* 1984; *accepted* 30 *October* 1984)

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