ACTA BIOL. SZEGED. 40, pp. 37-40. (1994)

EFFECTS OF ANTICHOLINESTERASES ON MUSCARINIC RECEPTOR BINDING PROPERTIES IN THE RAT BRAIN

Á. PÁLFI and K. GULYA*

Department of Zoology and Cell Biology, József Attila University, H-6701 Szeged P.O.B. 659, Hungary

(Received: July 1, 1994)

Abstract

Changes in the muscarinic acetylcholine receptor binding properties in response to *in vitro* administered cholinesterase (ChE) inhibitors were studied in rat brain homogenates. Several anticholinesterases (ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine, iso-OMPA and DFP) were tested to inhibit specific [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB) binding to rat brain membrane preparations. Under the conditions applied, the relative affinities of these compounds were found to decrease in the following sequence: ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine, iso-OMPA. DFP did not affect the specific binding of [^{3}H](-)QNB *in vitro*. The K_i values for the individual drugs were: ethopropazine, 10^{-7} M; 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, 3.1×10^{-6} M; eserine, 2.1×10^{-4} M; iso-OMPA, 10^{-3} M. The results suggest that certain ChE inhibitors might be able to compete with specific [^{3}H](-)QNB binding at the muscarinic receptor.

Key words: anticholinesterase, muscarinic acetylcholine receptor, CNS, rat

Introduction

Several studies have demonstrated that the number and pharmacological properties of the muscarinic acetylcholine receptors (mAChR) in nervous tissues change under various pathological conditions, such as disease (WASTEK and YAMAMURA, 1978; RUBERG et al., 1982), chronic administration of drugs (GAZIT et al., 1979; NOMURA et al., 1979; EHLERT et al., 1980a, b), or certain *in vitro* circumstances (AGUILAR et al., 1980). For example, the chronic administration of cholinesterase (ChE) inhibitors to animals led to behavioral tolerance (OVERSTREET et al., 1974). It has been suggested that this phenomenon may result from a decreased sensitivity of the mAChR in response to the increased levels of acetylcholine (ACh; RUSSEL et al., 1980). In other experiments (EHLERT and KOKKA, 1978; GAZIT et al., 1979), the chronic administration of organophosphates led to a marked reduction in the density of mAChR in several regions of the rat brain, as revealed by the binding profile of [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB). However, there relatively few

data are available concerning about the *in vitro* action of anticholinesterases on mAChR in the mammalian nervous system.

The main goal of the present study was therefore to investigate the *in vitro* effects of some of the most commonly used ChE inhibitors on the mAChR binding in the rat brain.

Materials and Methods

Male rats (CFY strain) weighing 180-200 g were used. The brains were quickly removed and homogenized (10% w/w) in ice-cold 0.32 M sucrose containing 0.1 mM EDTA in a glass homogenizer with a motor-driven Teflon pestle (6,000 rpm). The method used to study the binding of [^{3}H](-)QNB was a modification (GULYA and KÁSA, 1984) of that of YAMAMURA and SNYDER (1974). Briefly, the binding assay was performed in 50 mM sodium phosphate buffer (pH 7.4 at 25 °C) containing 0.5 nM [^{3}H](-)QNB (1.18 Tbq mmol^{-1}; Radiochemical Centre, Amersham, UK) in the presence or absence of various concentrations (10^{-11} to 10^{-3} M) of anticholinesterases. A second set was also prepared additionally containing 1 μM atropine, to determine the specific binding. The binding reaction was initiated by the addition of 50 μl of 0.1 % homogenate, and the incubation was allowed to proceed for 120 min at 25 °C. The incubation was terminated by rapid filtration of the mixture through a Whatman GF/C glass fiber filter. Each filter was washed with 5 ml of 50 mM sodium phosphate buffer (pH 7.4 at 4 °C) and then air-dried in scintillation vials. Ten ml of scintillation fluid (1000 ml of toluene, 150 mg of POPOP and 4 g of PPO) was added to each vial. The radioactivity was determined with an LKB 1215 Rackbeta II scintillation counter with 44% efficiency. Corrections for quenching were composed via a quench curve prepared by means of the external standard channel ratio method. The specific binding of [^{3}H](-)QNB was defined as the difference between the total and the nonspecific binding of the radioligand in the presence of 1 μM atropine. IC_{50} and n_H values were determined by indirect Hill (logit-log) plots of the inhibition of the specific [^{3}H](-)QNB binding by ChE inhibitors (GraFit 3.0, Erithacus Software, U. K.), and converted to K_i values via the equation $K_i=IC_{50}/(1+c/K_D)$, where c is the concentration of the radiolabeled ligand. Protein concentrations were determined by the method of LOWRY et al. (1951), using bovine serum albumin as standard.

The following ChE inhibitors were used: eserine sulfate, 10-[2-(dimethylamino)propyl]phenothiazine (ethopropazine hydrochloride), 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, tetraisopropyl-pyrophosphoramide (iso-OMPA), all from Sigma, St. Louis, USA, and diisopropyl-fluorophosphate (DFP; Fluka AG, Buchs, Switzerland). All drugs were freshly dissolved in 50 mM sodium phosphate buffer (pH 7.4 at 25 °C).

Results

The anticholinesterase agents were tested for their ability to displace specific [^{3}H](-)QNB binding to rat brain homogenate. The most potent inhibitor of specific [^{3}H](-)QNB binding among the anticholinesterases tested was ethopropazine (Table 1). Ethopropazine inhibited specific [^{3}H](-)QNB binding with a K_i value of about 10^{-7} M, while 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide, eserine and iso-OMPA had K_i values of 3.1×10^{-6}, 2.1×10^{-4} and 10^{-3} M, respectively. DFP at concentrations ranging from 10^{-11} to 10^{-3} M failed to affect mAChR binding.

Table I: Inhibition of [^{3}H](-)QNB binding by anticholinesterases. The concentrations required to inhibit [^{3}H](-)QNB binding to the receptors by 50% (IC_{50}) were determined from log probit plots and converted to K_i values via the equation $K_i=IC_{50}/(1+c/K_D)$, where c is the concentration of [^{3}H](-)QNB), and K_d is its dissociation constant.

Drugs	K_i (M)	n_H
atropine	10^{-10}	0.97
ethopropazine	10^{-7}	0.85
1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide	$3.1x10^{-6}$	0.70
eserine	$2.1x10^{-4}$	0.80
iso-OMPA	10^{-3}	0.84

Discussion

We have shown that, beside their known inhibitory effects on acetylcholinesterase (AChE, EC 3.1.1.7), anticholinesterases can inhibit specific [^{3}H](-)QNB binding to mAChR as well. Although several reports have previously investigated the effects of ChE inhibitors, relatively few of them demonstrate the *in vitro* effects on the binding characteristics of the receptor. GAZIT et al. (1979) showed that chronic administration of Tetram reduces the number of mAChR in several areas of the rat brain, while UCHIDA et al. (1979) reported a decreased [^{3}H](-)QNB binding in the ileum of the rat after DFP treatment. DAWSON and JARROTT (1981), however, could not demonstrate changes in the pharmacological properties of the mAChR in the brain and ileum as a result of administration of this drug. SIVAM et al. (1983) described that *in vivo* chronic administration of DFP reduces the number of mAChR sites without affecting their affinity, but *in vitro* treatment fails to affect the mAChR binding. In contrast, EHLERT and KOKKA (1978) and EHLERT et al. (1980a) reported that chronic DFP treatment decreased the [^{3}H](-)QNB binding in the striatum; however, they emphasized that this decrease was not due to a direct effect of DFP on the mAChR, since no inhibition of binding was produced by high concentrations of DFP added *in vitro*.

In our experiments, DFP did not influence the specific binding of [^{3}H](-)QNB *in vitro*, but another organophosphorus compound examined, iso-OMPA, did have a slight effect. The ChE inhibitors which compete with the binding of [^{3}H](-)QNB *in vitro* were ethopropazine, 1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide and eserine. The most potent of these was ethopropazine, which is in good agreement with earlier reports of its weak atropine-like effect (SILVER, 1974).

ChE inhibitors are widely used in physiological and pharmacological experiments in order to detect the effects of ACh electrophysiologically. SZERB and SOMOGYI (1973) were able to show that, when the AChE activity of rat cortical slices was inhibited with eserine, the release of ACh evoked by electrical stimulation was slightly depressed. As our results reveal, this may be due to the direct competitive effect of the anticholinesterase drug on the pre- and postsynaptic mAChR of cortical

cholinergic neurons and draws attention to the limits of its applicability in physiological experiments.

References

AGUILAR, J.S., CRIADO, M. and De ROBERTIS, E. (1980): Inhibition by local anesthetics, and propranolol of [^{3}H](-)quinuclidinyl benzilate binding to central muscarinic receptors. - Eur. J. Pharmacol. *68*, 317.

DAWSON, R.M. and JARROTT, B. (1981): Response of muscarinic cholinoceptors of guinea pig brain and ileum to chronic administration of carbamate or organophosphate cholinesterase inhibitors. - Biochem. Pharmacol. *30*, 2365.

EhlErt, F.J. and KOKKA, N. (1978): Decrease in [^{3}H](-)quinuclidinyl benzilate binding in the striatum of rats following chronic administration of diisopropylfluorophosphate. - Fed. Proc. *37*, 609.

EHLERT, F.J., KOKKA, N. and FAIRHURST, A.S. (1980): Altered [^{3}H](-)quinuclidinyl benzilate binding in the sitriatum of rats following chronic cholinesterase inhibition with diisopropylfluorophosphate. - Mol. Pharmacol. *17*, 24.

EHLERT, F.J., KOKKA, N. and FAIRHURST, A.S. (1980): Muscarinic receptor susceptibility in the longitudinal muscle of the rat ileum following chronic anticholinesterase treatment with diisopropylfluorophosphate. - Biochem. Pharmacol. *29*, 1391.

GAZIT, H., SILMAN, I. and DUDAI, Y. (1979, Administration of an organophosphate causes a decrease in muscarinic receptor levels in rat brain. - Brain Res. *174*, 351.

GULYA, K. and KÁSA, P. (1984): Transport of muscarinic cholinergic receptors in the sciatic nerve of the rat. - Neurochem. Int. *6*, 123.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951): Protein measurement with the Folin phenol reagent. - J. Biol. Chem. *193*, 265.

NOMURA, Y., KAJIYAMA, H., NAKATA, Y. and SEGAWA, T. (1979): Muscarinic cholinergic binding in striatal and mesolimbic areas of the rat: reduction by 6-hydroxydopa. - Eur. J. Pharmacol. *58*, 125.

OVERSTREET, D.H., RUSSEL, R.W., VASQUEZ, B.J. and DAGLISH, F.W. (1974): Involvement of muscarinic and nicotinic receptors in behavioral tolerance to DFP. - Pharmacol. Biochem. Behav. *2*, 45.

RUBERG, M., PLOSKA, A., JAVOY-AGID, F. and AGID, Y. 1982): Muscarinic binding and choline acetyltransferase activity in Parkinsonian subjects with reference to dementia. - Brain Res. *232*, 129.

RUSSEL, R.W., OVERSTREET, D.H., COTMAN, C.W. V.G., CHURCHILL, L., DAGLISH, F.W. and VASQUEZ, B.J. (1975): Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase, diisopropylfluorophosphate. - J. Pharmacol. Exp. Therap. *192*, 73.

SILVER, A. (1974): The biology of cholinesterases (North-Holland, Amsterdam), p. 471.

SIVAM, S.P., NORRIS, J.C., LIM, D.K., HOSKINS, B. and HO, I.K. (1983): Effect of acute and chronic cholinesterase inhibition with diisopropylfluorophosphate on muscarinic, dopamine, and GABA receptors of the rat striatum. - J. Neurochem. *40*, 1414.

SZERB, J.C. and SOMOGYI, G.T. (1973): Depression of acetylcholine release from cerebral cortical slices by cholinesterase inhibition and by oxotremorine. - Nature New Biol. *241*, 121.

UCHIDA, S., TAKEYASU, K., MATSUDA, T. and YOSHIDA, H. (1979): Changes in muscarinic acetylcholine receptors of mice by chronic administrations of diisopropylfluorophosphate and papaverine. - Life Sci. *24*, 1805.

WASTEK, G.J. and YAMAMURA, H.I. (1978): Biochemical characterization of the muscarinic cholinergic receptor in human brain: alternations in Huntington's disease. - Mol. Pharmacol. 14, 768.

YAMAMURA, H.I. and SNYDER, S.H. (1974): Muscarinic cholinergic binding in rat brain. - Proc. Natl. Acad. Sci. (Wash.) *71*, 1725.