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SHORT COMMUNICATIONS

In vitro effects of various cholinesterase inhibitors on acetyl- and butyrylcholinesterase of healthy volunteers

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Cholinesterase inhibitors have been widely used during the past decade in the treatment of (pre-)senile dementia of Alzheimer's type. As the progressive memory dysfunction is associated with a decreased rate of formation of acetylcholine in the brain, it might be useful to inhibit acetylcholine hydrolysis in order to restore synaptic function. Non-selective inhibitors of cholinesterase, however, have a low therapeutic index, and the inhibition of butyrylcholinesterase in plasma might contribute to adverse peripheral effects [1–4]; enzyme inhibitors with a higher affinity for acetylcholinesterase than for BuChE would thus be preferable to unselective ones. Testing the enzyme inhibiting potencies could support further research and development towards a therapeutic application in man. A radiometric method [5] has been used in this study to measure the reversible inhibitory influence exerted on human cholinesterases *in vitro* by physostigmine, pyridostigmine and tacrine, which are currently in both experimental and clinical use. As the activities of acetylcholinesterase in plasma and butyrylcholinesterase in erythrocytes are negligible [6, 7], the method does not require selective inhibition of distinct types of cholinesterases for determination of the respective enzyme. Selective inhibitors of acetyl- (BW 284C51) and butyryl- (iso-OMPA) cholinesterase have been used in this study, however, to test our assay for the reported selectivity [7–11].

Materials and Methods

Measurement of catalytic activity. The activity of BuChE in plasma and AChE in erythrocytes was measured at 25° as previously described [5, 12] without dilution of the samples using [^{14}C]acetylcholine iodide (NEN, Dreieich, F.R.G.), radiolabelled in the acetyl moiety, at final substrate concentrations of 3.6 mmol/L for AChE and 9.1 mmol/L for BuChE. The specific radioactivities were 418 kBq/mmol for AChE and 167 kBq/mmol for BuChE. Erythrocytes were haemolysed by freezing and thawing three times using liquid nitrogen. Samples were adjusted to pH 7.4 prior to incubation.

Concentration–response experiments. Concentration–response trials were performed as previously described [13]. Blood samples were drawn from healthy volunteers, collected in heparinized plastic tubes and shaken immediately. The whole blood was centrifuged for 15 min at 2200 *g*, and plasma and erythrocytes were separated before the addition of inhibitor. The following ones were used: BW 284C51 (Wellcome, Beckenham, U.K.); physostigmine (Serva, Heidelberg, F.R.G.); pyridostigmine (Mestinon™, Roche, Grenzach-Wyhlen, F.R.G.); tacrine (Serva) and iso-OMPA (Sigma, Deisenhofen, F.R.G.). After pre-incubation of the sample with the inhibitor for either 90 min (pyridostigmine only) or 60 min at 25° *in vitro*, the catalytic reaction was started by the addition of substrate.

Results

In our assay the specific BuChE inhibitor iso-OMPA exerted a 14 times more potent effect on human butyrylcholinesterase than on acetylcholinesterase. The optimal concentration of iso-OMPA to discriminate AChE from BuChE activity was 0.1 mmol/L, as might be seen from Fig. 1. The research compound BW 284C51, on the other hand, which is known as a specific inhibitor of AChE, was approximately 2100 times more potent in its effect on acetyl- than on butyrylcholinesterase and 0.01 mmol/L concentrations of BW 284C51 separated BuChE from AChE activity most specifically (data not shown). IC_{50} values for the inhibition of AChE and BuChE activity by iso-OMPA and BW 284C51 are given in Table 1.

Human acetylcholinesterase in erythrocytes has been inhibited using the reversible enzyme inhibitors physostigmine, pyridostigmine and tacrine. Physostigmine has been identified as the most effective inhibitor, followed by pyridostigmine and the less potent tacrine, with a similar slope of the concentration–response curve by each of these compounds, as demonstrated in Fig. 2. Human butyrylcholinesterase in plasma has proven to be most effectively inhibited by physostigmine, followed by tacrine and the less potent pyridostigmine. The respective IC_{50}

Table 1. Effects of various cholinesterase inhibitors

Inhibitor	N	IC_{50} AChE	IC_{50} BuChE	Selectivity on AChE	Selectivity on BuChE
BW 284C51	6	6.61×10^{-8}	0.14×10^{-3}	2118.00	0.0005
Galanthamine	8	3.55×10^{-7}	1.91×10^{-5}	53.80	0.02
Pyridostigmine	7	3.16×10^{-7}	8.51×10^{-7}	2.69	0.37
Physostigmine	7	2.45×10^{-8}	2.75×10^{-8}	1.12	0.89
Tacrine	8	1.26×10^{-6}	1.00×10^{-7}	0.08	12.6
iso-OMPA	8	0.85×10^{-3}	6.31×10^{-5}	0.07	13.5

Inhibitor concentrations causing half-maximal effect (IC_{50} [mol/L]) have been derived from a plot of per cent enzyme activity vs log concentration of inhibitor, while selectivities on acetylcholinesterase in erythrocytes and butyrylcholinesterase in plasma have been calculated as a ratio of the respective IC_{50} values.

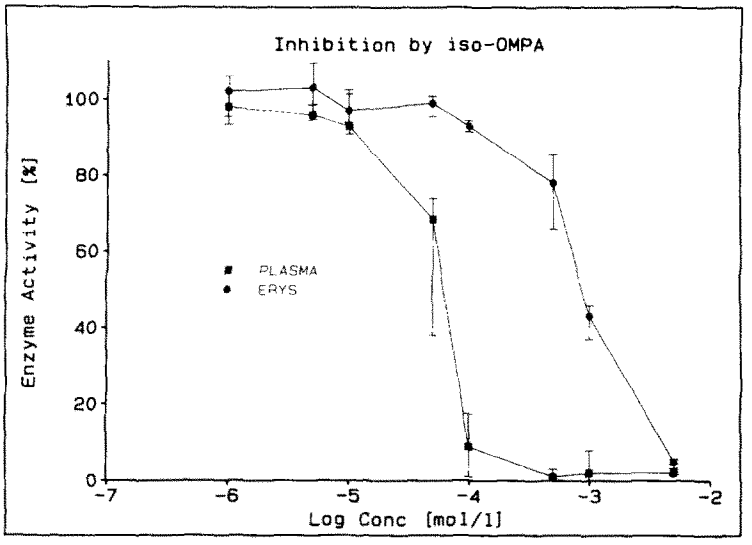

Fig. 1. Concentrations of between 1 μmol/L and 5 mmol/L of iso-OMPA were incubated for 1 hr at 25° *in vitro* with undiluted samples of human plasma (■) and erythrocytes (●) before the catalytic reaction was started by adding substrate. Values represent the median of enzyme activity of eight different blood samples from healthy volunteers and have been derived by calculating average values from duplicate assays run concurrently. Brackets indicate the first and third quartile of the data.

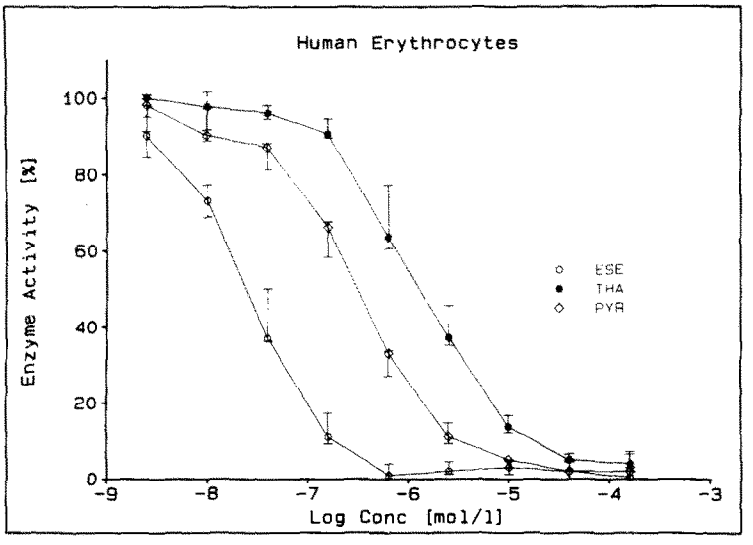

Fig. 2. Comparison of the inhibiting effects of physostigmine (ESE, ○), pyridostigmine (PYR, ◇) and tacrine (THA, ●) on acetylcholinesterase activity in human erythrocytes *in vitro*. The inhibitors were incubated for 60 min (ESE, THA) or 90 min (PYR) at 25° *in vitro* with undiluted samples of erythrocytes. Values represent the median of enzyme activity of seven (ESE, PYR) or eight (THA) different blood samples from healthy volunteers and have been derived and displayed as stated in Fig. 1.

value for the inhibition of AChE and BuChE activity by either one of these inhibitors are given in Table 1, while the slope of the concentration–response curve was similar to that with AChE in Fig. 2, and curves are therefore not displayed. Selectivity on AChE and BuChE activity by either one of the inhibitors used has been calculated on the basis of IC_{50} values derived from a plot of per cent enzyme activity versus log inhibitor concentration and the results are summarized in Table 1: BuChE activity was most selectively inhibited by iso-OMPA and tacrine, while AChE activity was specifically depressed by BW 284C51 and pyridostigmine.

Discussion

The specific inhibition of AChE by BW 284C51 and of BuChE by iso-OMPA has been used to separate these enzymes in brain homogenate and liquor samples [7–11]. If AChE activity is to be detected, BuChE might be suppressed using iso-OMPA. The optimal iso-OMPA concentration found in this study to inhibit BuChE *in vitro*

without altering AChE activity as well is in accordance with that reported by Sirviö *et al.* [10]. However, as iso-OMPA is only moderately specific (Table 1), its use is critical, as a 10-fold increase of the most specific concentration inhibited 50% of AChE activity (Fig. 1). In the assay of Lehmann and Fibiger [8], concentrations of 0.01 mmol/L inhibited almost 100% of BuChE only, while those of 0.1 mmol/L iso-OMPA resulted in an additional 30% inhibition of AChE activity. It should also be stressed that the range of enzyme inhibition varied to a greater extent with iso-OMPA than with BW 284C51. We would therefore suggest that, if iso-OMPA is used for separation purposes, the most specific concentration should be ascertained under the respective assay conditions. The optimal 'specific' concentration of BW 284C51 is in accordance with that specified by Brimijoin *et al.* [11], while Sirviö *et al.* [10] used 5.6 μmol/L, and Elble *et al.* [9] used 0.05 mmol/L concentrations of BW 284C51. However, although the selectivity of this acetylcholinesterase inhibitor is much higher than with iso-OMPA (Table 1), the slope of its concentration–response curve covers four orders of magnitude with either AChE and BuChE, and its use for separation of human cholinesterases is therefore equally critical: a 10-fold increase of the most specific concentration of BW 284C51 inhibited 40% of butyrylcholinesterase activity.

As the inhibition of BuChE has been shown to induce adverse peripheral effects and as the enhancement of the synaptic concentration of ACh in the brain is largely due to inhibition of the AChE activity, selective inhibitors of the AChE might turn out to be superior to non-selective inhibitors, such as physostigmine, in the treatment of Alzheimer's disease. Physostigmine and tacrine, both of which have been used in multiple clinical studies, seem to be less favourable than galanthamine (Nivalin™, Waldheim, Vienna, Austria), an alkaloid of the snowdrop. Galanthamine exhibited approximately 54-fold selectivity on human AChE activity *in vitro* (see Table 1 for comparison) and first data of human *in vivo* studies confirm that it might inhibit up to 65% of the AChE activity, without altering BuChE [12]. Galanthamine was reported to be well tolerated [14], which might be related to the lack of BuChE inhibition, as stated in the introduction. Double-blind, placebo-controlled trials are needed, however, to test its efficacy in Alzheimer patients. Except for the research compound BW 284C51, which is not available for use in humans, only pyridostigmine displayed selectivity for acetylcholinesterase but is inappropriate for Alzheimer patients due to its quaternary structure hampering central permeability. In conclusion, it is suggested to test further compounds for their enzymatic properties with human enzymes *in vitro* and *in vivo* and giving preference to drugs with selectivity for AChE.

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* To whom correspondence should be addressed.

Institut für Klinische Pharmakologie
Freie Universität Berlin
Klinikum Steglitz
Hindenburgdamm 30
D-1000 Berlin 45
Germany

T. THOMSEN*
B. ZENDEH
J. P. FISCHER
H. KEWITZ

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