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THE EFFECT OF SYMPATHOLYTIC AND SYMPATHOMIMETIC AGENTS ON ACETYLCHOLINESTERASE AND CHOLINESTERASE ACTIVITY, *IN VITRO*

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Abstract—Alpha blocking drugs competitively inhibited AChE in rat blood cells and brain homogenates but beta receptor blocking compounds were ineffective up to a concentration of 10^{-3} M. The alpha blocking activity of the drugs does not correlate with their AChE inhibitory effect. Two compounds structurally similar to the sympathetic alpha blocking drugs but without any blocking effect on the receptors inhibited AChE non-competitively. Only some of the sympathomimetic agents inhibited AChE. A few of the compounds tested also influenced serum ChE activity but their ability to inhibit AChE and ChE was not parallel.

Some of the alpha blocking agents have been reported to inhibit cholinesterase (pChE; EC 3.1.1.8., acylcholine acylhydrolase) and acetylcholinesterase (AChE; EC 3.1.1.7., acetylcholine acetylhydrolase) activity [1, 2, 3]. Boyd *et al.* [4] demonstrated that yohimbine and piperoxane had the same effect. The kinetics of AChE inhibition produced by dibenamine and phenoxybenzamine have been investigated by Beddoe and Smith [5]. They demonstrated two different types of inhibition: (1) an alkylating reaction at about pH 9; (2) another mechanism at neutral pH ranges. The inhibitory effect of epinephrine and norepinephrine on AChE has also been reported [6].

The pChE inhibiting effect of some beta blocking drugs has been published recently [7]. However, no comparative study is known on the effect of different alpha and beta blockers and sympathomimetics on AChE and pChE activity at physiological pH ranges.

MATERIALS AND METHODS

Rat red blood cells (0.2 ml/tube), serum (0.1 ml/tube) and brain homogenate were used as enzyme source. The whole brain was removed and homogenised in distilled water (20%, w/v) in a Potter homogeniser and diluted for the assay to 3.3 g wet wt/l. Butytrylcholine iodide was used as a substrate for pChE and acetyl-β-methyl-choline chloride for AChE determination. The enzyme activity was measured by a modified Hestrin method [8, 9].

The following compounds were tested: epinephrine (Tonogen, Richter), norepinephrine bitartrate (Richter), *N*-isopropyl-noradrenalin (Euspyran, Spofa), Synephrin tartrate (Sympathomim, EGYT), *l*-phenylephrine HCl (Sigma), ergotamine tartrate (Richter), ergometrine maleate (Richter), dihydroergotoxine (Redergam, Richter), methysergid (Deseril, Sandoz), phentolamine methanesulfonate (Regitine, Ciba), tolazoline HCl (Ciba), phenoxybenzamine, dibenamine, propranolol (Inderal, I.C.I.), alprenolol (Aptin, Haessle), pronethalol (Alderlin, I.C.I.), I.C.I. 50172, pindolol (Visken, Richter), oxprenolol (Trasicor, Ciba).

To evaluate the inhibitory effect of the compounds several dilutions were tested from the minimally effective concentrations up to 10^{-3} M. The dose–response curves were plotted on a semilogarithmic scale and the ID_{50} (the concentration of the inhibitor needed to produce 50 per cent inhibition of the enzyme) was determined by interpolation. The results were expressed in pID_{50} i.e. $-\log ID_{50}$ (M). Concentrations of epinephrine, phenylephrine and phenoxybenzamine higher than 10^{-3} M were also tested. Pindolol was used between 2×10^{-4} and 10^{-5} M. The kinetic analysis was performed by the method of Lineweaver and Burk [10].

RESULTS AND DISCUSSION

pID_{50} values are given in Table 1. The type of AChE inhibition was estimated with red blood cells. All of the alpha blocking agents tested in our experiments inhibited AChE and the type of inhibition proved to be competitive. Generally, the tested substances gave similar pID_{50} values for both the red blood cell and brain homogenate enzymes.

Some of the sympathomimetic agents also inhibited AChE, but neither isoproterenol nor Synephrin was effective and *l*-phenylephrine inhibited AChE activity only at high concentrations.

A few of the tested drugs also influenced pChE activity, but their ability to inhibit AChE and pChE was not parallel. For instance, ergotamine proved to be a potent AChE inhibitor, but it was ineffective on pChE. The alpha blocking activity of the tested drugs does not correlate with their AChE inhibitory effect.

None of the beta receptor blocking compounds tested inhibited AChE activity up to a concentration of 10^{-3} M.

Two compounds, structurally similar to the sympathetic alpha blocking drugs but without any blocking effect on the receptors (ergometrine and methysergid) were also tested. They inhibited AChE, but the type of inhibition was non-competitive.

The high concentrations of the drugs needed for the *in vitro* inhibition do not necessarily mean that

Table 1. The effect of some sympathomimetic and sympatholytic agents on acetylcholinesterase and cholinesterase activity

Inhibitors	Red blood cell AChE pID_{50}	Brain AChE pID_{50}	Serum pChE pID_{50}	Type of inhibition (red blood cell AChE)
Epinephrine	3.1	2.8	—	—
Norepinephrine	3.1	3.1	3.0	competitive
N-Isopropyl-noradrenaline	0	0	0	—
Synephrin	0	0	0	—
l-Phenylephrine	2.7	—	—	—
Dibenamine	3.5	—	—	competitive
Phenoxybenzamine	3.3	2.9	0	competitive
Tolazoline	4.2	3.9	3.9	competitive
Phentolamine	3.3	3.2	3.1	competitive
Ergotamine	4.2	4.3	9	competitive
Dihydroergotoxine	4.3	3.9	4.4	competitive
Ergometrine	3.3	(?) 0	0	non-competittive
Methysergid	3.8	3.9	3.5	non-competitive
Propranolol	0	0	3.3	—
Pronethalol	0	0	0	—
Alprenolol	0	0	3.4	—
Oxprenolol	0	0	3.9	—
Pindolol	0	0	3.7	—
I.C.I. 50172	0	0	0	—

the doses of epinephrine or alpha blocking agents usually administered *in vivo* would inhibit acetylcholinesterase. However, they might exert an additive effect on cholinesterase inhibitors. The use of these substances in the therapy of alkylphosphate poisoning therefore seems to be hazardous.

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