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Pharmacological evaluation of phenyl-carbamates as CNS-selective acetylcholinesterase inhibitors

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Summary. The pharmacological and clinical properties of a novel phenyl carbamate acetylcholinesterase (AChE) inhibitor, SDZ ENA 713 are described. In animals and human subjects this compound showed superior chemical stability, oral bioavailability and a longer duration of action than physostigmine. SDZ ENA 713 produced a 10-fold greater inhibition of AChE in the hippocampus and cortex than in the heart and skeletal muscle, which explains its relatively low toxicity and freedom from cholinergic side effects. The selective effect in the cortex and hippocampus may be due to its preferential inhibition of the G1 form of the enzyme, which is present in relatively higher concentrations in these brain areas. Evidence of a selective hippocampal action was obtained in normal human subjects in whom REM sleep density was increased at doses that had no effect on plasma cholinesterase. If memory impairments in AD are related to a lack of cholinergic activity in cortical and hippocampal brain areas, SDZ ENA 713 should produce significant symptomatic improvement.

Introduction

Alzheimer's dementia (AD) is a severe degenerative disorder in which there is selective damage to neurons in the forebrain and hippocampus (Whitehouse et al., 1981). Although this results in significant changes in several neurotransmitters, the reduction in the number of cholinergic neurons and in choline acetyl-transferase, correlates best with the memory impairment (Sims and Bowen, 1983). Anticholinergic drugs also cause memory disturbances and produce some of the symptoms of early dementia in normal human subjects (Drachmann and Leavitt, 1974). Thus, in the absence of a more specific treatment for AD, acetylcholinesterase (AChE) inhibitors have been tested in this and other forms of dementia in the hope that they would increase cholinergic transmission in the affected brain areas and improve cognitive function. In one study, which reported some beneficial effect with physostigmine, the extent of the improvement was correlated

with the degree of cholinesterase inhibition in the spinal fluid and thus to the amount of the drug reaching the CNS (Thal et al., 1983). However, the narrow therapeutic window, unpredictable oral bioavailability and high incidence of side effects of physostigmine, resulting mainly from peripheral cholinergic hyperactivity (Christie et al., 1981), prompted the search for safer AChE inhibitors with a more selective effect in the CNS.

Derivatives of N-N-dimethylamino-ethyl-phenyl carbamate

For this purpose we prepared a series of N-monoalkyl and N,N-dialkyl derivatives of m-[1-(N,N-dimethylamino)-ethyl] phenyl carbamate, many of which are more lipid soluble and show greater chemical stability than physostigmine (Weinstock et al., 1986). Their inhibitory activity was evaluated in vitro on a purified preparation of human erythrocyte AChE, and on whole brain AChE after subcutaneous injection to mice. The concentration of each drug that would inhibit by 50% the activity of the enzyme preparation was determined by the method of Ellman et al. (1961). From ex vivo measurements, the dose of each drug was also computed that would block total brain enzyme activity by 50% (ED_{50}), when this reached its peak after injection (Weinstock et al., 1986, 1992). The most potent inhibitors of the human erythrocyte enzyme were the mono- and dimethyl derivatives. Increasing the length of the alkyl chain to ethyl resulted in an 80-fold reduction in inhibitory potency in both mono- and di-alkyl derivatives. This was gradually restored in the former, to values similar to that of the methyl derivative, as the chain lengthened from n-propyl, through n-butyl to n-hexyl. In general, this relationship between chemical structure and anti-AChE potency was maintained in the whole animal, the exception being the l-isomer of the methyl-ethyl derivative, SDZ ENA 713, (formerly RA7, Weinstock et al., 1986) which was about 10 times more potent in vivo than would be expected from its activity on red cell AChE (Weinstock et al., 1992).

Acute toxicity of phenylcarbamates

Acute toxicity was assessed after sc. injection of the carbamates in mice. Therapeutic ratios (LD_{50}/ED_{50}) were derived, where LD_{50} is the dose that is lethal in 50% of mice. The monomethyl, dimethyl and methyl-ethyl derivatives were the least toxic. Increasing the chain length, or introduction of a branched chain, e.g. isopropyl or sec-butyl resulted in greater toxicity (Weinstock et al., 1992).

Death from overdose of anti-AChE is due to respiratory arrest, which itself results from a combination of excessive stimulation of muscarinic receptors in the respiratory centre in the brainstem and paralysis of the respiratory muscles (depolarization blockade via nicotinic receptors) (Machne and Unna, 1963). The former can be prevented by the admini-

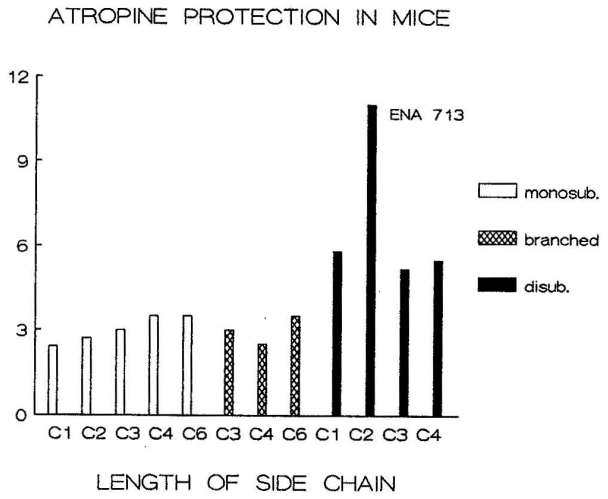

Fig. 1. Increase in LD_{50} of SDZ ENA 713 by pretreatment of mice with atropine. Ordinate: Ratio of LD_{50} in the presence and absence of atropine (5 mg/kg) Abcissa: No. of C atoms in alkyl substituent on carbamate. Open columns: monosubstitued; filled columns: disubstituted; crossed hatched columns; branched side chain. * SDZ ENA 713

stration of a centrally-active muscarinic antagonist such as atropine or scopolamine. On the other hand, muscle paralysis cannot be antagonised by drugs. The relative contributions of excess central muscarinic receptor stimulation and muscle paralysis towards the lethal effects of anti-AChE were assessed by comparing the acute toxicity of the phenyl carbamates with and without pretreatment by atropine (5 mg/kg). Muscarinic receptor blockade resulted in a 2–3.5-fold increase in LD_{50} in physostigmine and in all the mono-alkyl and branched-chain derivatives. This rose to 5–6-fold in the dimethyl, methyl-propyl and methyl-butyl derivatives and to 12-fold in the methyl-ethyl compound, SDZ ENA 713 (Fig. 1). This finding suggested that SDZ ENZ 713 inhibited brain AChE at much lower dose levels that those needed to paralyse the respiratory muscles and was therefore a potentially much safer drug than the other derivatives or physostigmine.

AChE inhibition in different regions of rat brain

After oral administration to rats, SDZ ENA 713 caused a preferential inhibition of AchE in the cortex and hippocampus, in comparison with other brain regions. This is shown in Fig. 2. The relatively weak inhibition in the heart and skeletal muscle, that was suggested by the atropine protection experiment, can readiliy be seen in the same animals (Table 1). In contrast, physostigmine inhibited the enzyme in all brain areas and in the periphery at similar doses. Direct measurements of the concentrations of ACh produced in the CNS by increasing doses of SDZ ENA 713, also

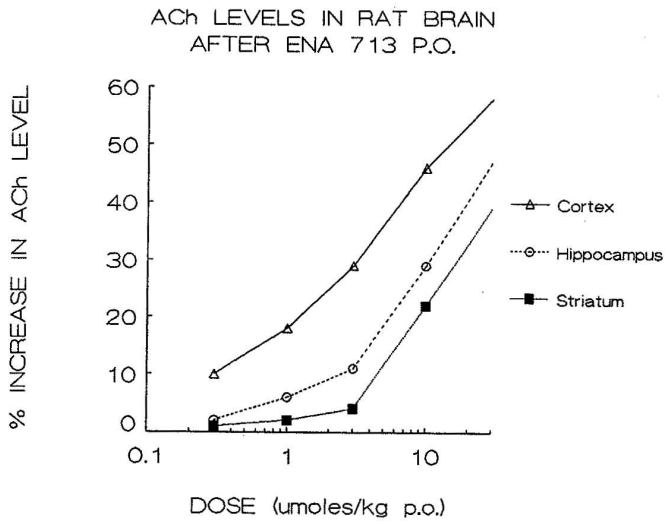

Fig. 2. Inhibition of AChE in different regions of rat brain by SDZ ENA 713. Brains were removed 30 min after oral administration of the drug or saline. Inhibition of AChE represented as % of value in saline treated controls

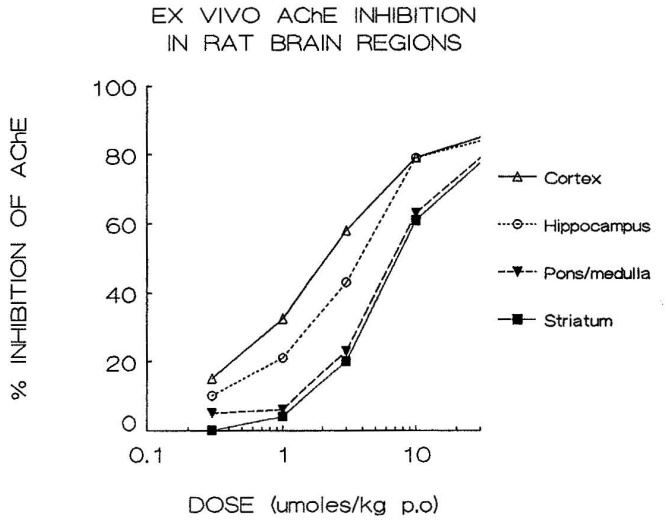

Fig. 3. Increase in acetylcholine levels in different brain regions by SDZ ENA 713. Measurements were made 30 min after oral administration of the drug or saline. Levels of acetylcholine expressed as a % of saline control

confirmed a preferential increase in the cortex and hippocampus, which correlated well with the AChE inhibition (Fig. 3).

The reason for the relatively greater inhibition of AChE in the cortex and hippocampus than in the striatum or medulla is not yet clear. However,

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