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Diphasic EEG Effects Induced by Anticholinesterasic Agents in Mice

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Acetylcholine esterase (AChE) inhibitors at toxic doses induce profound biochemical alterations in the brain of animals, and also induce changes in motor behavior, learning and memory, REM sleep, vision, audition and thermoregulation (Karczmar, 1977).
However, biochemical effects exerted by these drugs in general show a poor correlation to behavioral effects (Bignami et al, 1975; Lehotzky, 1982). Such a relationship is even less evident when behavioral effects induced by low doses of AChE inhibitors which are unable to produce clear biochemical alterations, are taken in consideration. This therefore suggests that low doses of AChE inhibitors may induce effects which are not on cholinergic pathways.
Wolthuis and Vanwersh (1984), using low doses of physostigmine, induced in rats a performance reduction at shuttle box and open field - both tests requiring integrity of neuromotor functions. Such a behavior has been put in tight relationship to the theta 1 type rhythm recorded from electroencephalographic activity (EEG) in the hippocampus in rodents, and theta 1 type rhythm has been correlated to biochemical mechanisms other than cholinergic ones. For these reasons we have attempted to assess quantitatively, with the aid of computerized analysis, the activity induced by low doses of AChE inhibitors, physostigmine and paraoxon, on the theta 1 type rhythm in the EEG of mice.
The effects induced by both substances were also confronted to assess whether they could induce dose-related effects on the theta 1 type rhythm, which were different from those attributed to AChE inhibition, and in order to make evident possible differences in their mechanism of action.

MATERIALS AND METHODS

Forty-four DBA/2 male mice weighing 22 to 24 grams were chronically implanted with four cortical electrodes (anterior and posterior sensorimotor cortex). EEGs were recorded on paper and on magnetic tape according to the following schedule: a control EEG was recorded for two consecutive hours; then, mice were injected with saline, 0.2 ml i.p.; EEG was again recorded for 2-3 minutes after 5 and then after 15 minutes, and thereafter every 15 minutes after treatment for at least 2 hours. On the following day, a recording session was performed with

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the same animals, which were also treated with paraoxon, in doses of: 0.025, 0.05, 0.1, 0.2, 0.4 mg/kg, while another group of animals were treated with physostigmine in doses of : 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg, four animals per dose. Each animal was treated only once with the drug.
The EEG signal was band-pass filtered 0.5-42 Hz and sampled offline, power spectrum analysis was performed, and after visual inspection, consecutive artifact-free power spectra, ranging from 0 to 63.5 Hz with 0.5 Hz discrimination , were stored on digital tape. The results are presented using a function model for relative power of the frequency band 7.5-12 Hz, which corresponds to theta 1 type rhythm (for details see Zapponi et al, 1978; Loizzo et al, 1985).

RESULTS AND DISCUSSION

After low doses of physostigmine and paraoxon (up to 0.1 mg/kg), no gross behavioral alterations were noted; while higher doses of physostigmine (0.8-0.4 and sometimes 0.2 mg/kg) and paraoxon (0.4 mg/kg) induced transitory hypomobility and horripilation . On visual inspection, EEG records relative to the control and to animals treated with both substances (0.025 to 0.1 mg/kg) appeared normal. In two animals out of four treated with an intermediate dose of paraoxon (0.2 mg/kg) and in all animals treated with higher doses of paraoxon (0.4 mg/kg) and physostigmine (0.4-0.8 mg/kg), visual inspection of EEG records showed transitory slowing in frequency and diminution in amplitude, although the morphology of the EEG waves was not affected. No clear signs of seizures were ever noted.
Computerized analysis showed that the smallest doses of physostigmine and paraoxon (0.025 and/or 0.05 mg/kg, equivalent to 1.5-5% of LD 50) induced an increase in power of the 7.5 to 12 Hz frequency band of the spectrum . This increase, however, was not significant. Doses of 0.1 to 0.8 mg/kg induced a dose-dependent decrease in power of the 7.5-12 Hz frequency band (Fig. 1 and 2).
Such results show that both drugs induce a diphasic effect : i.e., lower doses produce an increase in power, while higher doses induce a highly significant, dose-related reduction of power of the 7.5-12 Hz frequency band . This effect was accompanied by a diminution of motor activity.
There is much discussion about the origin and role of the theta 1 type rhythm. In many studies of neurophysiology, it is considered to be linked to behaviors such as walking, running, rearing, etc., and not directly dependent on acetylcholine activity. Various authors (for references see Bland, 1986) reported that effects induced by physostigmine do not have any clear connection to mechanisms involving the inhibition of AChE; other authors report connection to mechanisms involving neuropeptides, dopamine (Coudray-Lucas et al, 1983) and GABA (Sivam et al, 1983). Some hypothesize the existence, at septal areas, of a pacemaker which triggers theta 1 type rhythm (Bland, 1986). GABAergic projections to the hippocampus may also originate here (Kohler et al, 1984).
Dose/effect curves drawn in Fig.3 show outstanding similarity between the two substances in the expression of cerebral electric signal, thus indicating very similar mechanisms for physostigmine and paraoxon.
The dose/effect relationship in both substances show an increase in

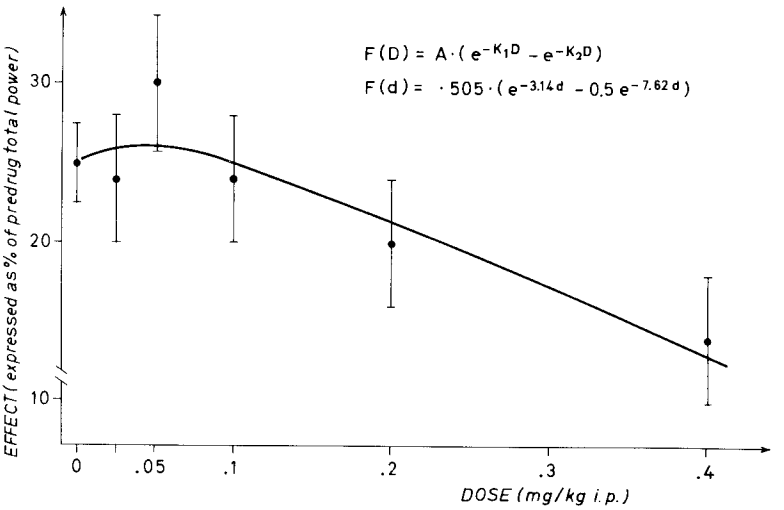

Figure 1. Exponential functions interpolated from 7.5-12 Hz frequency band of spectrum power values in the first hour after i.p. administration of paraoxon . Each point represents mean values plus or minus the standard deviation (4 animals per dose).

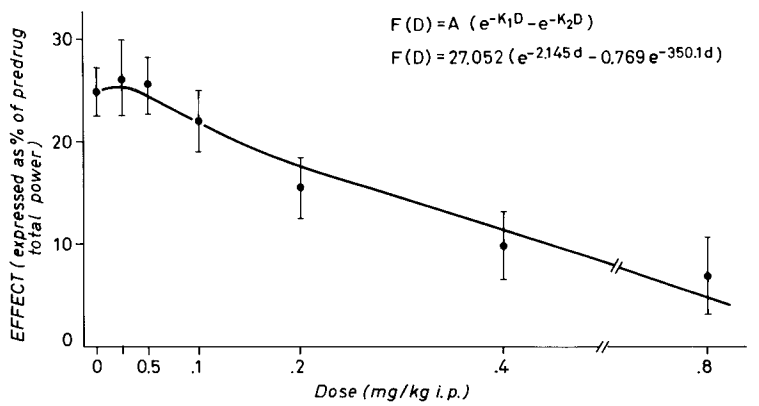

Figure 2. Exponential functions interpolated from 7.5-12 Hz frequency band of spectrum power values in the first hour after i.p. administration of physostigmine . Each point represents mean values plus or minus the standard deviation (4 animals per dose).

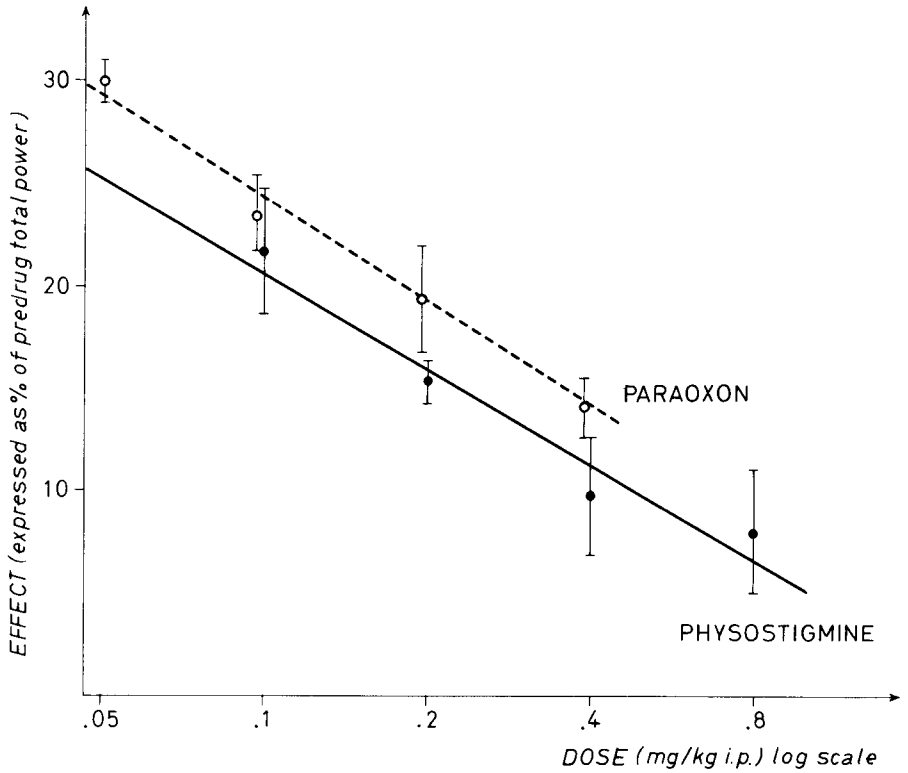

Figure 3. Regression curves relative to effects induced by paraoxon versus physostigmine on EEG in mice. Normalized power value of 7.5-12 Hz band during the first hour after i.p. administration (four animals per dose).

theta 1 type rhythm, followed by a consistent decrease. This diphasic behaviour suggests that the biochemical mechanism elicited by the drugs may be either due to the interference to two biochemical systems (one of the two probably being of cholinergic origin), or to the interference exerted, directly or indirectly, on one biochemical system different from the cholinergic one.

According to Goldberg (1965), physostigmine administered i.p. at doses of 0.16, 0.32, 0.64, and 1.28 mg/kg, in rats, can reduce in a dose-dependent manner, the cerebral AChE levels (to 82, 72, 67 and 45 per cent of the control values, respectively). Our results indicate that very similar doses induce, in mice, a significant, dose-dependent reduction on the power of theta 1 type rhythm; therefore, biochemical effects of physostigmine, although obtained in different genera of animals, seem to fit quite well with neurophysiological models. At lower doses (0.025-0.05 mg/kg), for which no consistent biochemical effects are reported in the literature, physostigmine and paraoxon induce a slight increase on the 7.5-12 Hz band power. This latter

result seem to mirror behavioral data reported by Pradhan and Mhatre (1970) indicating an increase in the motor activity of rats treated with 0.05 mg/kg of physostigmine. We can suggest, therefore, that at high doses of AChE inhibitors biochemical effects are paralleled by EEG analysis, while biochemical to EEG relationship may be not evident at lower doses.
At the end, this study may be a useful contribution for establishing an analytical method of EEG in mammals aimed to surveilling the health of workers chronically exposed to anticholinesterasic substances at doses that are not able to produce clear clinical symptoms or significant biochemical alterations.

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