Effects of four cholinesterase inhibitors on the response of the guinea-pig isolated ileum to transmural electrical stimulation

Cholinesterase inhibitors have been used to preserve acetylcholine released from isolated tissues, despite the fact that some (eserine and neostigmine) may cause acetylcholine to be released and others (dyflos and mipafox) may not adequately protect released acetylcholine (Cox, Hecker & Weston, 1970; Cox & Lomas, 1972). If the organophosphorus inhibitors are inefficient in protecting released acetylcholine, then it would be expected that they would give a poor potentiation of acetylcholine released by transmural electrical stimulation. If the reversible inhibitors give good protection to released acetylcholine, then they would be expected to give good potentiation of acetylcholine released by electrical stimulation. Therefore the effects of eserine, neostigmine, dyflos and mipafox have been tested on the guinea-pig isolated ileum, stimulated either electrically or by the addition of acetylcholine to the tissue bath.

The ileum was set up for isometric recording at 37° in Krebs aerated with 5% carbon dioxide in oxygen. Log concentration and log frequency effect lines (transmural stimulation 20–60V, 0·3 ms pulse width, 0·2–200 Hz) were obtained on the same piece of ileum before and after exposure to one of the cholinesterase inhibitors. Concentrations of inhibitor were chosen that gave similar potentiation of exogenous acetylcholine without affecting the resting tone of the ileum. Shifts in the effect lines produced by the inhibitors were measured at the 80% response level. This was necessary because, when potentiation occurred, the response to a single electrical pulse was equivalent to the original 50% response. In these instances there was also an increase in the maximum response and the lines were parallel between the 50 and 90% responses. The results of the experiments are shown in Table 1.

All four inhibitors produced similar leftward shifts in the acetylcholine log concentration effect lines. When dyflos and mipafox were used shifts in the frequency effect lines were significantly less than shifts in the concentration effect lines ($P < 0{\cdot}001$). When eserine and neostigmine were used there was no significant difference between the shifts ($P > 0{\cdot}05$).

Thus, endogenous acetylcholine released by transmural stimulation was, as predicted, poorly potentiated by the organophosphorus inhibitors. A probable explanation for this finding is that acetylcholine released by transmural stimulation comes predominantly from the myenteric plexus (Paton & Zar, 1965), an area rich in acetyl-

Table 1. *Mean shifts in log concentration and log frequency effect lines (± standard error) produced by four cholinesterase inhibitors on the guinea-pig isolated ileum preparation, minimum number of observations for each mean = 8.*

Inhibitor	Concentration (g/ml)	Mean shift in effect lines: Exogenous acetylcholine	Mean shift in effect lines: Transmural stimulation
Eserine	10^{-8}	0·93 ± 0·11	0·88 ± 0·21
Neostigmine	10^{-8}	0·69 ± 0·15	1·07 ± 0·12
Dyflos	10^{-6}	0·97 ± 0·15	—0·20 ± 0·18*
Mipafox	10^{-8}	0·88 ± 0·09	0·13 ± 0·12

* Negative sign indicates net antagonism.

cholinesterase (Ambache, Freeman & Hobbiger, 1969). The reversible inhibitors are efficient acetylcholinesterase inhibitors (Augustinsson, 1948) and therefore potentiate. The organophosphorus inhibitors are less efficient acetylcholinesterase inhibitors (Aldridge, 1953) and therefore do not potentiate.

These results would support the contention that the organophosphorus inhibitors are of little value in experiments designed to collect and assay acetylcholine released from isolated tissues. A further possibility, that they may also suppress acetylcholine release, has yet to be explored.

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Do "loser" rats become "winners"?

It was reported by Masur, Karmiol & Neto (1972) that *Cannabis sativa* induces winning behaviour in previous loser rats. We have some evidence which make us doubt the justification of these terms.

In our experiment—done according to the description of Grossmann & Grossman (1970) rats were trained to go through a tunnel to obtain food. Some animals were trained from right to left, others on the reverse direction. Once in each session of 10 trials, two animals were brought into the tunnel at opposite ends at the same time and the one that pushed the other animal backwards out of the tunnel more than three successive times, we named "winner" rat.

The antianxiety drug diazepam, made "loser" rats, "winners" (Table 1). However, close observation showed that the lack of defensive postures and the perseverant "stubborn" behaviour of these rats was the reason that the former "winner" rats finally gave up and withdrew.

We concluded that we were observing the effect of drug-induced loss of social responsiveness in a situation where the limited space reduced the possibilities of provoked fighting. Maybe the interference with the normal ecological patterns of submission in drugged animals would explain this surprising result as well as such apparent contradictory observations as the taming effect on the one hand and the increased fighting on the other hand, induced by anxiolytic drugs and by cannabis derivatives (Ten Ham, 1972; Carlini & Masur, 1969; Guaitani, Marcucci & Garrattini, 1971).

Under these circumstances the terms "loser" and "winner" are perhaps inappropriate. To substantiate our conclusion, we repeated the experiment with a limited number of rats, who after four days experience of encountering a live rat, were faced with a small plug of cotton wool in the tube. Though this plug could be pushed away with a minimum pressure, rats who forced live rats to withdraw did so themselves from