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A selective potentiation by naloxone of L-dopa but not atropine suppression of oxotremorine-induced tremor in mice

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Oxotremorine-induced tremor activity in mice was suppressed by pretreatment with either L-dopa or atropine. Additional pretreatment with the opiate receptor blocker naloxone significantly potentiated the antitremor effect of L-dopa but not that of atropine. These findings indicate a selectivity of drug interaction between naloxone and L-dopa.

Parkinsonism represents a neurologic deficit in which there is an imbalance between dopaminergic and cholinergic mechanisms in the basal ganglia (Klawans 1973). Post-mortem examination of brains of parkinsonian patients has revealed a marked deficiency of striatal dopamine, which results in an uncountered cholinergic dominance that is responsible for the tremor, rigidity and akinesia that is characteristic of parkinsonism. Pharmacotherapeutic management of parkinsonism has generally followed one of two basic approaches: the reduction of the cholinergic dominance, i.e. treatment with centrally acting muscarinic receptor blockers, or enhancement of central dopamine function, i.e. treatment with the dopamine precursor L-dihydroxyphenylalanine (L-dopa). Oxotremorine-induced tremor activity in mice represents a simple and useful animal model of parkinsonism, since both atropinic drug treatment and L-dopa can reduce the incidence and severity of the tremor (Everett et al 1956; Bebbington & Brimblecombe 1965; Korczyn & Eschel 1979). Previous research in our laboratory has shown that pretreatment with the opiate receptor blocker naloxone can potentiate the antitremor effect of L-dopa in this paradigm (Quock & Lucas 1983). The present study was conducted to determine whether pretreatment with naloxone might also potentiate atropine suppression of oxotremorine-induced tremor in mice.

Methods

Male ICR mice (King Animal Laboratories, Oregon, Wisconsin), 20–30 g, were used. Tremor was induced by an oxotremorine challenge and assessed by the method of Huang et al (1980). Animals were preselected for ability to balance for at least 60 s on horizontal wooden dowels, 15 cm in length and 12 mm in diameter, projecting from a vertically mounted particleboard at a height of 20 cm. Tremor activity was assessed as the tendency of oxotremorine-challenged animals to fall off the dowels. Significant tremor was judged to be present if the mouse failed in three attempts to maintain its balance on the dowel for the minimum 60 s. While this particular behavioural test might not distinguish between drugs that induce tremor and those that produce central nervous system (CNS) depression, this was not a concern in our study as the oxotremorine precursor tremorine, and presumably also oxotremorine, possesses CNS stimulatory, not CNS depressant, properties (Chen & Bohner 1958). Experiments were conducted between 1200 and 1600 h in a well-illuminated laboratory at an ambient temperature of 24 ± 1 °C.

Drugs used were oxotremorine (Sigma), 1-β-dihydroxyphenylalanine methyl ester hydrochloride (Sigma), atropine sulphate (Sigma) and naloxone hydrochloride (DuPont). Oxotremorine, which came in the liquid base form, was diluted to appropriate concentration in double distilled water. Solutions containing L-dopa, atropine and naloxone were also prepared in double distilled water. Doses for L-dopa, atropine and naloxone repesent the weights of their respective salts, while doses for oxotremorine indicate the weight of the base. L-Dopa and atropine were administered intraperitoneally 30 min and naloxone subcutaneously 5 min before the intraperitoneal oxotremorine challenge. The injection volume for all drugs was 0·1 ml per 10 g weight. Various control groups received subcutaneous or intraperitoneal injections of distilled water in lieu of drug.

Animals of all treatment groups were tested for tremor activity at 15, 30, 45 and 60 min following oxotremorine challenge. In this test, the cumulative percentage of animals falling off the dowels during the four test periods was recorded and the degree of tremor activity in each group was quantified by determining the mean effective dose (ED50) of oxotremorine for that group, i.e. that dose of oxotremorine which would produce significant tremor in 50% of the mice in a given group. The oxotremorine ED50 value and 95% confidence intervals of each treatment group were determined according to Litchfield & Wilcoxon (1949). Antagonism of oxotremorine-induced tremor activity was reflected as an increase in the ED50 value of oxotremorine for that group. Potentiation of the antitremor effect of either L-dopa or atropine was determined by a further increase in the ED50 value of oxtremorine for that group. Potency ratios were used to

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determine statistically significant differences between ED50 values of different groups ($P < 0.05$).

Results

Table 1 compares the ED50 values and 95% confidence intervals of oxotremorine challenge in different groups of animals subjected to various pretreatments. Oxotremorine produced dose-dependent tremor activity that was unaltered by pretreatment with naloxone at a dose of 10 mg kg^{-1}. Pretreatment with L-dopa at a dose of 300 mg kg^{-1} antagonized tremor activity as evidenced by a significant elevation of the ED50 value of oxotremorine. In animals pretreated with L-dopa and naloxone, there was a further significant increase in the ED50 value, indicating a potentiation by naloxone of the antitremor effect of L-dopa. Based on preliminary dose-response experiments, we ascertained that atropine at a pretreatment dose of 0·3 mg kg^{-1} would also antagonize tremor activity and increase the ED50 value of oxotremorine by approximately the same degree as L-dopa at a dose of 300 mg kg^{-1}. However, in animals pretreated with atropine and naloxone, there was no further change in the ED50 value, suggesting that naloxone had no influence upon the antitremor effect of atropine.

Table 1. The influence of naloxone upon the antitremor effect of L-dopa and atropine in oxotremorine-challenged mice. All mice were pretreated with distilled water or L-dopa (300 mg kg^{-1}) or atropine (0·3 mg kg^{-1}) at −30 min; distilled water or naloxone (10 mg kg^{-1}) at −5 min; and oxotremorine challenge at time 0. The data represent the oxotremorine ED50 values (μg kg^{-1}) with 95% confidence intervals.

Treatment group	Oxotremorine ED50 (μg kg^{-1}) (95% confidence intervals)	Potency ratio for Antagonism	Potency ratio for Potentiation
First experiment			
Oxotremorine	142·5 (130·2–156·0)	—	
Naloxone + oxotremorine	141·4 (126·6–157·9)	0·99	
L-Dopa + oxotremorine	166·8 (151·6–183·4)	1·17*	—
L-Dopa + naloxone + oxotremorine	213·4 (183·7–247·9)	1·50*	1·20*
Second experiment			
Oxotremorine	139·6 (126·7–153·7)	—	
Naloxone + oxotremorine	140·1 (130·3–150·5)	1·00	
Atropine + oxotremorine	167·0 (139·8–199·4)	1·19*	—
Atropine + naloxone + oxotremorine	165·5 (141·7–193·2)	1·19*	0·99

N of at least 24 animals per group. *$P < 0.05$, compared to oxotremorine group for antagonism or to L-dopa/oxotremorine or atropine/oxotremorine group for potentiation.

Discussion

These findings indicate a specificity of naloxone for L-dopa in potentiation of antitremor drug effect. It is also significant to note that naloxone alone failed to suppress tremor activity, suggesting that perhaps naloxone must interfere with some tonic opiate receptor stimulation in order to cause potentiation. Earlier studies have demonstrated that opiate receptors are located on striatal dopamine nerve terminals and that opiate receptors are possibly inhibitory to dopamine neuronal function (Pollard et al 1978; Schwartz et al 1978). We have observed that pretreatment with the tyrosine hydroxylase-inhibitor α-methyl-*p*-tyrosine can prevent naloxone potentiation of apomorphine-induced stereotypic climbing in mice, one explanation for which is that endogenous dopamine might be required for naloxone potentiation (Quock et al 1984). There is also evidence that dopamine and apomorphine can enhance the efflux from rat striatal slices of methionine-enkephalin, an effect that is reversed by neuroleptic drugs (Pasinetti et al 1984). We are presently investigating the possibility that dopamine receptor stimulation may activate some endogenous opiate mechanism that inhibits dopamine neuronal activity. Blockade of the opiate receptors may disinhibit the neuron, resulting in an increased outflow of transmitter and enhanced dopamine receptor stimulation. This may be the explanation why naloxone potentiates L-dopa but not atropine suppression of oxotremorine-induced tremor in mice.

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