MODIFICATION OF THE TOXICITY OF ACETYLCHOLINE BY PRIOR ATROPINIZATION IN MICE

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Summary: The potentiation of acetylcholine (Ach) toxicity in mice with prior atropinization was tested. The experiments were carried out with three doses of 200 *mg*, 300 *mg*, and 400 *mg/kg* of Ach administered ip. Prior atropinization was observed to potentiate the Ach toxicity at all dose levels of atropine except the highest in the group that received Ach 200 *mg/kg* and the results were variable in the other two groups.

Key words: prior atropinization acetylcholine-toxicity potentiation

INTRODUCTION

The cholinergic activity of atropine on the isolated guinea pig ileum has been reported by Ashford *et al.* (1). These workers have also demonstrated potentiation of acetylcholine (Ach) toxicity in mice with suitable doses of atropine. Lullman *et al.* (6) have shown that atropine possesses anticholinesterase activity. The cholinergic activity of hyoscyamine has been observed on the frog rectus muscle by Teitel (7). Ashford *et al.* (1) used only one dose of Ach (200 *mg/kg* ip) in their studies. The present communication describes observations made on the influence of prior atropinization in three groups of mice given three different doses of Ach.

MATERIALS AND METHODS

The experiments were carried out in mice of either sex weighing between 16-25 *g*. Groups of 40-45 animals each received 200 *mg/kg*, (Group I), 300 *mg/kg* (Group II), or 400 *mg/kg* (Group III) of the chloride salt of Ach (control groups).

Atropine sulphate was given in doses of 1 *mg*, 0.1 *mg*, 0.01 *mg*, 0.001 *mg*, 0.0001 *mg* and 0.00001 *mg/kg* in groups of 10-20 animals each, 15 min prior to the administration of Ach as detailed above. The volume of injection did not exceed 1 *ml* in any experiment. The required dilutions were prepared in normal saline from the 1 % stock solution. Both Ach and atropine were administered ip. The animals were observed for mortality for 30 min after Ach administration.

RESULTS

In group I, the highest dose of atropine (1 *mg/kg*) and in groups II and III, the two highest doses of atropine (1 *mg* and 0.1 *mg/kg*) blocked Ach toxicity. In group I the lowest dose of atropine tested (0.00001 *mg/kg*) produced potentiation as also the four intermediate doses, 0.1 to 0.0001 *mg/kg*. In groups II and III the lowest dose, 0.00001 *mg*/kg produced blockade (Table I).

Table I: Percentage mortality in mice with Acetylcholine alone and with Acetylcholine given 15 min after atropine.

Group No.	*Acetylcholine chloride mg/kg*	*Atropine sulphate mg/kg*	*Total No. of mice*	*Mean % died*	*Effect*	*Level of significance of difference from control (P value)*	*P values for potentiation (Pooled data)**
I	200	—	45	13.3	—		
	,,	1	10	10	Block	<.9	
	,,	0.1	15	26.67	Potentiation	<.5 *	
	,,	0.01	10	30	Potentiation	<.3	<.1
	,,	0.001	10	30	Potentiation	<.3	
	,,	0.0001	10	30	Potentiation	<.3	
	,,	0.00001	20	30	Potentiation	<.2	
II	300	—	40	25	—		
	,,	1	10	0	Block	<.2	
	,,	0.1	10	10	Block	<.7	
	,,	0.01	10	50	Potentiation	<.3 *	
	,,	0.001	10	50	Potentiation	<.3	<.05
	,,	0.0001	10	50	Potentiation	<.3	
	,,	0.00001	10	10	Block	<.7	
III	400	—	40	57.5	—		
	,,	1	10	30	Block	<.2	
	,,	0.1	10	40	Block	<.5	
	,,	0.01	10	80	Potentiation	<.5 *	
	,,	0.001	10	60	Potentiation	<.8	<.2
	,,	0.0001	10	80	Potentiation	<.5	
	,,	0.00001	10	50	Block	<.9	

*The data for potentiation was also pooled and analysed statistically, probabilities for this analysis are shown in the last column.

DISCUSSION

The observations in group I confirm those reported by Ashford *et al.* (1) that potentiation of Ach toxicity (200 *mg/kg* ip) occurs by prior atropinization with small doses, (0.0001 *mg/kg* and 0.00001 *mg/kg*). These workers had observed blockade of Ach toxicity with atropine in doses of 1 *mg/kg*, 0.1 *mg/kg*, 0.01 *mg/kg* and 0.001 *mg/kg* though complete blockade was observed only with the highest two doses. In the present work, in group I some blockade occurred only with the highest dose of atropine.

The variability in the influence of prior atropinization on subsequent Ach effect has been reported for the isolated guinea pig ileum by Ashford *et al.* (1). These workers observed blockade of Ach contractions with higher doses of atropine, potentiation with intermediate doses and no effect with the lowest doses tested in their series. However, in the present work, experiments in group II and III bring out an interesting phenomenon of blockade reappearing with very low doses of atropine.

Atropine is a known parasympathetic blocking agent. The potentiating action of an antagonist in low concentrations has also been observed with adrenoreceptor blocking agents, dibena-

mine and phenoxybenzamine (3, 4, 5). Potentiation of Ach toxicity observed by Ashford *et al.* (1) has been explained by a possible anticholinesterase activity of atropine (6). Teitel (7) has also reported cholinergic activity of hyoscyamine on frog rectus with low concentrations (3.16×10^{-17}M to 3.16×10^{-14}M and some activity upto 3.16×10^{-11} M) and its absence with higher (3.16×10^{-10}M) and lower concentrations (3.16×10^{-20}M).

From the observations of the present work and that of the other workers cited above, potentiation is seen only with lower doses of atropine. In the present data the differences from the control are not statistically significant by a stringent test of significance ($P<0.05$). Nevertheless, many of the differences which are obvious and significant only by a less stringent test i.e. $P < 0.2$ and $P < 0.1$ could merit consideration (2). Furthermore, in group II pooled data for potentiation was significant ($P<0.05$).

The blockade of Ach with high doses of atropine is an accepted phenomenon. It is the occurrence of potentiation or variability in action with lower doses which requires discussion. It is possible that atropine in very low doses diffuses more easily into the biophase of the receptors or across the cell membranes and also has greater accessibility to the receptors and the inactivating enzyme system. Thus, with low doses there may occur either blockade of Ach actions by occupation of the cholinergic receptors or potentiation by inhibition of the inactivating enzymes. The resultant effect would depend upon which of these two actions is dominant. There may also occur absence of any effect if the two actions neutralize each other.

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