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CHOLINERGIC MODULATION OF HEXOBARBITAL HYPNOTIC ACTION IN THE MOUSE

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ABSTRACT

Pilocarpine (10mg/kg, s.c.) enhanced the hypnotic action of hexobarbital (50mg/kg, i.p.) and the hypnotic action of barbital 300mg/kg, i.p.) in male mice when given 20 min prior to the given barbiturate. These pilocarpine induced enhancements of barbiturate hypnotic action were reversed by atropine sulfate (20mg/kg, i.p.), given 20 min prior to the pilocarpine, but not by atropine methyl bromide (20mg/kg, i.p.), given 20 min prior to the pilocarpine, analogously. Arecoline (40mg/kg, s.c.), given 20 min prior to hexobarbital (50mg/kg, i.p.), caused extension of hexobarital hypnotic action when given subsequent to atropine methyl bromide (20mg/kg, i.p.) administered 20 min previously but not when atropine sulfate (20mg/kg, i.p.) was administered in place of the atropine methyl bromide, analogously. The pilocarpine enhancement of hexobarbital action, as influenced by the atropine methyl bromide, was not significantly different from that which obtained when that anticholinergic was not given. The pilocarpine enhancement of barbital action, when influenced by the atropine methyl bromide, was slightly greater than the extension produced when such influence was absent. These results suggest that the cholinergic agent induced enhancements of barbiturate action observed are effects exerted by those agents on the central nervous system.

INTRODUCTION

Prior findings have demonstrated extension of barbiturate and phenothiazine tranquilizer action induced by anticholinesterase agents under conditions which best support the concept that a central nervous system (CNS) cholinergic action is involved in these anticholinesterase effects[5, 7, 8]. It has also been shown that pilocarpine and arecoline, two receptor active cholinergic agents with CNS activity, can extend the action of phenothiazine tranquilizers[4], and that pilocarpine can extend the action of ethyl alchol[6]. In both of these cases the extension produced was reversed by the CNS active anticholinergic agent, atropine surfate, but not by the predominantly peripherally active anticholinergic agent, atropine methyl bromide. These reports have engendered our interest in the question as to whether or not pilocarpine and arecoline would exert effect on the duration of hexobarbital hypnotic action in a manner which was different when any such effect was influenced by atropine methyl bromide and atropine sulfate, respectively. We have also become interested in whether or not barbital sodium hypnotic action would be affected in a manner similar to that of hexobarbital under such conditions. It was reasoned that test of barbital sodium in this analogous way would afford insight with regard to possible influence of biotransformation on any of the observations made since hexobarbital elimination *in vivo* is considerably involved with biotransformation[9] while the elimination of barbital sodium *in vivo* is not[1]. The present study was undertaken in order to continue and extend investigations concerned with the modulation of the actions of some central nervous system depressants by cholinergic agents.

METHODS AND MATERIALS

Adult albino Swiss ICR male mice obtained from ARA Sprague-Dawley (Madison, Wisconsin), weighing 20-26g, were used as experimental animals. Animals were housed 10/cage in no. 101 keystone plastic cages lined with Pel-E-Cel bedding (Paxton Processing Co., Paxton, Illinois). Under a 12 hour on, 12 hour off lighting cycle, they were allowed to acclimate to the laboratory environment for 2-5 days prior to use. The laboratory temperature was maintained at 23-24°C. The mice had access to Purina rodent chow and tap water *ad libitum*.

Pilocarpine was used as the hydrochloride and arecoline as the hydrobromide. The anticholinergies used were atropine sulfate and atropine methyl bromide. Hexobarbital was used as the sodium salt prepared by dissolving 50mg of the acid form in 4.2ml of 0.05 N NaOH, and diluting with distilled water to make a concentration of 5.0mg/ml (pH 10.5 - 11.5). Barbital was employed as the sodium salt. All drugs were obtained from the Sigma Chemical Co., St. Louis, Mo.

The atropinium agents and the barbiturates were given intraperitoneally. Pilcarpine and arecoline were administered subcutaneously. Intraperitoneal administrations were given in a volume of 0.01ml/g body weight; subcutaneous administrations were made at a volume of 0.005ml/g body weight. Atropine derivatives were given at 40 min and the cholinergic agents at 20 min prior to the given barbiturate administration, respectively. All injections were made using a lml turberculin syringe fitted with a 27 gauge, 0.5 in needle. In a given experiment, each animal, whether treated or control, received the same number and volume of injections, by analogous routes of administration and on similar time schedule, using physiological saline as "sham" injection where necessary. For each mouse loss of righting reflex time induced by the barbiturate was measured. Loss of righting reflex after barbiturate administration was assumed to be present when a mouse, exhibiting immobility in the upright posture, was unable to right itself when unrighted 3 successive times with a 30-second period of testing for the loss of righting reflex.

Return of the righting reflex was deemed to be present when the mouse, having exhibitied loss of righting reflex, spontaneously righted itself and maintained its righting ability when unrighted 3 times within the 30-second immediately following such spontaneous righting. The data obtained in this way was used to calculate group mean values which were subjected to student's t-test for assessment of the statistical significance between differences in mean values observed. Probability values at or less than the 5 percent level were accepted as significant in employing these tests.

Results

The results presented in Table 1 show the effects of 10mg/kg pilocarpine hydrochloride on the hypnotic effect of 50mg/kg hexobarbital sodium, when this cholinergic agent was given either alone or subsequently to either 20mg/kg atropine methyl bromide or 20mg/kg atropine sulfate. These findings indicate that under the conditions of the experiment employed, pilocarpine hydrochloride was capable of significantly extending the effect of the hexobarbital when given either alone (Group II) or following prior administration of atropine methyl bromide (Group III) but not when given subsequently to atropine sulfate administration (Group IV). The mean values providing these extensions were about 442 percent (Group II) and 390 percent (Group III) of control mean value (Group I), respectively. Difference in the mean values for pretreatment with pilocarpine given alone (Group II) from that when given subsequent to atropine methyl bromide (Group III) was not statistically significant.

Table 1 Effect of pilocarpine, given subsequent to atropine methyl bromide or atropine sulfate, on 50mg/kg hexobarbital hypnotic action

Group of 10 Mice	Drugs and dosages given prior to 50mg/kg hexobarbital sodium I.P.[a]	Loss of righting reflex time, (min) (Mean ± S.E.)[b]
I.	0.01ml/gm 0.9% NaCl (I.P.) 0.005ml/gm 0.9% NaCl (S.C.)	16.3 ± 2.7
II	0.01ml/gm 0.9% NaCl (I.P.) 10mg/kg Pilocarpine HCI (S.C.)	72.0 ± 2.3
III.	20mg/kg atropine MeBr (I.P.) 10mg/kg Pilocarpine Hcl (S.C.)	63.5 ± 3.5
IV.	20mg/kg atropine SO_4 (I.P.) 10mg/kg Pilocarpine HCl (S.C.)	18.5 ± 4.5

(a) Atropinium agents or I.P. saline were given at 40 min and the pilocarpine or S.C. saline at 20 min prior to the hexobarbital, respectively.

(b) P values from comparison of mean responses (calculated by t-test): Group I compared with either Group II or III, $p<0.001$; Group IV compared with either Group II or III, $p<0.001$; Groups I and IV, $p>0.1$; Groups II and III, $p<0.1>0.05$

The findings entered in Table II indicate the results obtained when an experiment was performed to investigate the effect of arecoline hydrobromide on hexobarbital hypnotic action when given subsequently to either atropine methyl bromide or atropine sulfate, respectively. Given subsequently to 20mg/kg atropine methyl bromide, 40mg/kg of arecoline hydrobromide caused extension of the hexobarbital action (Group II) in a comparison with action of that barbiturate in control animals (Group I). This extension was reflected in a mean value which was about 204 percent of control mean value. However, when given following prior administration of 20mg/kg atropine sulfate, the same dose of arecoline did not cause an extension of hexobarbital action (Group III).

Table III presents data obtained with the effect of pilocarpine hydrochloride on the hypnotic action of barbital sodium when given alone and when given following administration of either atropine methyl bromide or atropine sulfate, respectively. Given pursuant to 20mg/kg atropine methyl bromide, 10mg/kg of pilocarpine caused extension of the barbital sodium action (Group III), producing a mean loss of righting reflex time induced by 300mg/kg barbital sodium which was about 239 percent of the control mean value (Group I). Conversely, when given subsequently to 20mg/kg of atropine sulfate, the same dose of pilocarpine did not significantly extend the barbital sodium action (Group IV) in a comparison with the barbital action in control mice. Enhancement of barbital action caused by the pilocarpine when given pursuant to atropine methyl bromide (Group III) was slightly greater than that it produced when given alone (Group II).

Table II Effect of arecoline, given subsequent to atropine methyl bromide or atropine sulfate on 50mg/kg hexobarbital hypnotic action

Group of 10 Mice	Drugs and dosages given prior to 50mg/kg hexobarbital sodium I.P.[a]	Loss of righting reflex time, (min) (Mean ± S.E.)[b]
I.	0.01ml/gm 0.9% NaCl (I.P.) 0.005ml/gm 0.9% NaCl (S.C.)	15.7 ± 1.8
II.	20mg/kg atropine MeBr (I.P.) 40mg/kg arecoline HBr (S.C.)	32.0 ± 4.0
III.	20mg/kg atropine SO_4 (I.P.) 40mg/kg arecoline HBr (S.C.)	16.5 ± 2.5

(a) Atropinium agents or I.P. saline were given at 40 min and the arecoline or S.C. saline at 20 min prior to the hexobarbital respectively.

(b) P values from comparison of mean responses (calculated by t-test): Groups I and II, $p<0.01$; Groups II and III, $p<0.01$; Groups I and III, $p>0.1$

Table III Effect of pilocarpine, given subsequent to atropine methyl bromide or atropine sulfate, on 300mg/kg barbital hypnotic action

Group of 10 Mice	Drugs and dosages given prior to 300mg/kg barbital sodium, I.P.[a]	Loss of righting reflex time, (min) (Mean ± S.E.)[b]
I.	0.01ml/gm 0.9% NaCl (I.P.) 0.005ml/gm 0.9% NaCl (S.C.)	128.8 ± 7.0
II.	0.01ml/gm 0.9% NaCl (I.P.) 10mg/kg Pilocarpine HCl (S.C.)	250.2 ± 9.17
III.	20mg/kg atropine MeBr (I.P.) 10mg/kg Pilocarpine HCl (S.C.)	307.6 ± 23.5
IV.	20mg/kg atropine SO_4 (I.P.) 10mg/kg Pilocarpine HCl (S.C.)	157.7 ± 15.3

(a) Atropinium agents or I.P. saline were given 40 min and the pilocarpine or S.C. saline at 20 min prior to the barbital, respectively.

(b) P values from comparison of mean responses (calculated by t-test: Group I compared with either Group II or Group III, $p \ll 0.01$; Group IV compared with either Group II or Group III, $p<0.01$; Groups II and III, $p<0.05$; Groups I and IV, $p>0.1$

DISCUSSION

The experiments performed appear to demonstrate that under certain conditions pilocarpine can enhance the hypnotic action of hexobarbital and barbital in the mouse. Similar effect of arecoline on the hypnotic action of hexobarbital appears to have been shown, albeit that that effect is less than that of pilocarpine. These cholinergic agent enhancements of barbiturate action are apparently exerted when these agents are administered subsequent to prior administration of atropine methyl bromide but not when given following prior administration of atropine sulfate. The pattern of the influence of these two anticholinergic agents on the pilocarpine and arecoline modulations of barbiturate action would make it appear that this response to these cholinergic agents is more related to a CNS action than to a peripheral action of these drugs. The basis for this tentative conclusion derives from the fact that their effects were not yielded when influenced by atropine sulfate but were evident when influenced by atropine methyl bromide, the anticholinergic agents having been given under conditions so as to expect CNS anticholinergic activity from the former but not the latter[4, 8, 10]. A similar pattern governing the influence of these two anticholinergics on pilocarpine extension of ethanol action[6], and on pilocarpine and arecoline extension of phenothiazine tranquilizer action[4] has been shown to exist. In the latter instance and in our own work reported here the cholinergic agent enhancement of CNS depressant action seen was produced with more potency by pilocarpine than by arecoline. Pilocarpine has greater muscarinic potency than does arecoline and arecoline has greater nicotinic potency than does pilocarpine[2]. Those differences in potency at muscarinic and nicotinic sites, considered in conjunction with the fact that the cholinergic agent extension of barbiturate action seen in our results is more potently produced by pilocarpine than by arecoline, may indicate that this response is more related to muscarinic actions of these agents than it is to their nicotinic actions.

Other workers, using a strain of mice different from the one we used have obtained results showing pilocarpine induced extension of hexobarbital hypnotic action which they interpret as being contributed to by pilocarpine suppression of hepatic hexobarbital enzyme activity[11]. They also found that atropine methyl nitrate, which does not readily cross the blood-brain barrier, reduced the extension of hexobarbital action caused by pilocarpine to about the same extent as the reduction in that effect caused by atropine sulfate, which does penetrate into the brain well. This result is somewhat at variance with the findings which we are reporting here and would support the possibility of a peripheral action of pilocarpine being responsible for pilocarpine induced enhancement of hexobarbital action, at least in part. While our own findings presented here do not rule out a peripheral acting component of the action of pilocarpine in extending hexobarbital effect our results are most supportive of the proposition that a portion of the mechanism involved in this extension is located in the CNS. In the case of the pilocarpine induced extension of hexobarbital action which we observed, that extension was not significantly reversed when the pilocarpine was given following prior administration of atropine methyl bromide but was reduced to control level of hexobarbital action when given pursuant to atropine sulfate. The extending action of the pilocarpine on hexobarbital hypnosis when given alone was not significantly greater than that seen when it was given subsequent to atropine methyl bromide. A somewhat similar pattern of results was yielded when pilocarpine effect on barbital hypnotic action was investigated. Given alone, the pilocarpine extended the barbital action well above control level. This enhancement was not only not blocked by atropine methyl bromide, it was slightly greater under influence of that anticholinergic agent than without it. Conversely, atropine sulfate reversed this pilocarpine enhancement of barbital action. These findings are indicative of a muscarinic modulation of barbiturate action in the CNS which is atropinium agent sensitive. Causing extensions of hexobarbital and barbital action when given alone, the receptor-active muscarinic agent pilocarpine yielded such responses when influenced by atropine methyl bromide but not when influenced by atropine sulfate, under conditions so as to expect CNS anticholinergic activity from the latter agent but not the former one [4, 8, 10]. If peripheral activity of the pilocarpine made a marked contribution to its mechanism of action producing extension of barbiturate effect then one would expect to see significant decrease in that extension produced by atropine methyl bromide as well as by atropine sulfate if the pilocarpine effect in this respect was atropinium sensitive. Our results do not support such a possibility. Since the pattern of anticholinergic agent influence on pilocarpine induced extension of barbiturate action was the same in the case of barbital as it was in the case of hexobarbital, we have provided evidence supportive of a postulate which postulates that CNS activity of a muscarinic agent can modulate barbiturate action by extending that action irrespective of its peripheral factor influences, such as its effects on barbiturate pharmacokinetics and biotransformation[11]. Basis for this postulate is provided by the great similarity in the pattern of anticholinergic agent influence on pilocarpine effect which we obtained whether hexobarbital, a barbiturate markedly eliminated by biotransformation[9], or barbital, which is virtually not biotransformed at all[1], was the barbiturate with action extended by the pilocarpine. Finally, discrepancies which appear to exist between the findings of others, who have reported a significant contribution made by peripheral actions to pilocarpine extension of hexobarbital action[11], and our own results, which appear to minimize the role of such actions, may be well associated with strain influenced differences in the mice used by the workers involved. The well known strain differences in sensitivity to barbiturate action in mice which have been reported previously[3] give credence to this possibility and make for the probability that discrepancies between the findings of laboratories working this field are more apparent than real.

Finally, it should be noted the question of the mechanism of action. The mode of action of muscarinic receptors are coupled to biochemical effector systems that trigger second messengers (activation of C kinase by DAG, mobilization of calcium triphosphate).

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