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INFLUENCE OF SKF525-A ON THE BEHAVIORAL AND ANTICHOLINESTERASE EFFECTS OF CERTAIN CARBAMATES*

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Abstract—The effects of eserine, compound 10854 (3 isopropylphenyl-N-methyl carbamate), and carbaryl (1-naphthyl-N-methyl carbamate) on discrete avoidance behavior and brain cholinesterase inhibition were studied in rats. The influence of SKF525-A (β-diethylaminoethyl diphenylpropylacetate HCl) on the effects of these agents was investigated. When given alone at a dose of 25 mg/kg, SKF525-A did not alter behavioral response or brain cholinesterase levels. When it was given 30 min prior to eserine, a 4- to 8-fold increase in the number of shocks normally taken after eserine alone was observed with a similar enhancement of inhibitory effects of cholinesterase. Similar schedules with compound 10854 or carbaryl revealed about a 2-fold enhancement of the behavioral effects of these agents. Concomitant potentiation of anticholinesterase effects was obtained with compound 10854 but not with carbaryl. Animal which received SKF525-A and carbaryl showed no difference in cholinesterase levels from those given only carbaryl. It is suggested that carbaryl is converted to a metabolite, devoid of anticholinesterase activity, which undergoes further metabolism through microsomal enzyme involvement.

SKF525-A (β-diethylaminoethyl diphenylpropylacetate HCl) possesses minimal pharmacological activity alone but has the ability to enhance the activity of many therapeutic agents. Inhibition of drug-metabolizing enzymes present in liver microsomes and the soluble fraction of liver cells has been considered as the major mechanism in drug potentiation.[1, 2] The activity of certain compounds is antagonized by SKF525-A; notable among these are several organic phosphate anticholinesterase insecticides.[3] This effect has been attributed to conversion of some agents to active metabolites by microsomal enzymes. Thus SKF525-A has been demonstrated to be involved in the enzymatic conversion of many drugs, either by preventing metabolism to a nonactive or less active material or by preventing activation to the biologically active molecule. The subject of drug synergism with particular emphasis directed to SKF525-A and central nervous system depressants has recently been reviewed.[4] Previous studies from this laboratory have considered the psychopharmacological and reversible anticholinesterase activities of certain carbamates useful as insecticidal or therapeutic agents.[5, 6] Because of continued interest in the behavioral effects of cholinergic stimulation and because of the dichotomous effects of SKF525-A on drug

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activity, it was considered desirable to study the influence of this agent on the inhibitory effect on behavioral and cholinesterase actions of selected carbamates. The agents used were eserine, compound 10854 (3-isopropylphenyl-N-methyl carbamate), and carbaryl (Sevin,* 1-naphthyl-N-methyl carbamate).

METHODS

Discrete or noncontinuous avoidance behavior was studied in 10 male Carworth Farms Elias (CFE) rats, weighing between 375 and 450 g, which were allowed food and water *ad libitum* except during training and experimentation. Studies were performed in a 10-compartment rat shock box (Lehigh Valley Electronics) with white noise background. Each compartment, of 4·5 in × 8 in × 8 in, was supplied with one lever, which had an operating pressure of 12 g, a cue light, consisting of a 24 V bulb, and an individual grid floor to which 3 mA of current was delivered by means of a grid scrambler. Current was measured and controlled by means of an ammeter and a voltage adjustor. The box was operated by programming equipment with necessary timers, and relays and data were recorded on Sodeco counters.

Approximately 10% of CFE rats can be trained to 95% or better efficiency in avoiding shock, in sessions of 120 avoidance trials per hr with a session length of 2 hr. A 3-month training period is necessary to attain this criterion of efficiency. A schedule of 18-sec intertrial, 10-sec cue light, and 2-sec shock was used in which the animal could not terminate the shock by responding during it. Results are expressed as the average number of shocks taken by the group during a given period. Control sessions were scheduled on Monday, Wednesday, and Thursday and drug sessions on Tuesday and Friday. Behavioral response on control days never showed any carry-over effects from preceding drug tests.

Rat brain cholinesterase was determined by a modification of the method of Larsson and Hansen[7] in which the hydrolysis rate of acetylcholine bromide was followed by means of an automatic recording titrator at 38°.[8] Rats were killed by decapitation; the brains were removed and placed in a container surrounded by ice and processed in the cold. A 10% whole-brain homogenate in cold 0·25 M sucrose, prepared by using a motor-driven Teflon-Pyrex homogenizer, was centrifuged at 10,000 *g* for 15 min to remove most of the mitochondria and cell debris.[9] The supernatant containing microsomal and soluble enzyme was used. These fractions constitute almost 90% of cholinesterase activity in rat brain and are claimed to be almost entirely acetylcholinesterase.[9] Addition of alkali neutralized the liberated acid and maintained the pH at 7·4. Reactions were followed for 10 min, and enzyme activity was expressed as an activity slope, being defined as alkali consumption as a function of time.

The brain cholinesterase activity slopes for 60 saline-treated male rats weighing between 175 and 225 g was 152 ± 1·8 (S.E.) Preliminary results revealed no difference in sensitivity to the lethal or anticholinesterase effects of the carbamates between rats of this weight range and the heavier rats used in the behavioral studies. Previous time–response studies indicated that all three agents produced maximal inhibition of brain enzyme 30 min after intraperitoneal injection, coincident with their maximal effect on discrete avoidance behavior.

* Trademark of Union Carbide Corp., New York, N.Y.

All drugs were prepared just prior to use. Eserine was supplied as the salicylate sale, SKF525-A* as the hydrochloride; both were dissolved in saline. Compound 10854† was available as a 48% wettable powder which when added to saline produced a stable dispersion. Carbaryl† was used as the pure chemical and was dissolved in a 50% aqueous solution of polyethylene glycol 400. Extensive preliminary work revealed no behavioral or anticholinesterase effects from this solvent. Drug concentrations were so varied as to administer them in a constant volume proportional to the weight of the animals. Dosages reported refer to the amount of pure compound 10854 or carbaryl given or of the salts of eserine and SKF525-A; carbamate doses were selected from previous dose-response curves. Depending upon the degree of variances, Student's "t" test or Cochran and Cox "t" test was used for comparison of treatment means.[10]

SKF525-A was given 30 min prior to the carbamates in all cases. In behavioral studies, carbamates were administered intraperitoneally 15 min after the start of an experimental session.

RESULTS

Behavioral studies were performed in block arrangements. After establishing that SKF525-A at a dose of 25 mg/kg i.p. did not alter normal avoidance response or brain cholinesterase activity, the animals were given the anticholinesterase agent alone. On

TABLE 1. INFLUENCE OF SKF525-A ON THE BEHAVIORAL EFFECTS OF ESERINE, COMPOUND 10854, AND CARBARYL*

Treatment (dose, mg/kg)	Cumulative min after inj.	Average number shocks ± S.E.		Difference†
		Alone	With SKF525-A	
Controls‡	30	2·4 ± 0·3	2·6 ± 1·3	Not signif.
	120	9·2 ± 1·3	10·3 ± 3·9	Not signif.
Eserine (0·16)	30	6·1 ± 2·0	25·4 ± 7·9¶	$P < 0.01$
	120	12·7 ± 3·7	94·0 ± 26·7¶	$P < 0.01$
Compound 10854 (0·5)	30	8·3 ± 3·9§	17·4 ± 6·0¶	$P < 0.05$
	120	14·3 ± 5·1	34·6 ± 12·5¶	$P < 0.01$
Carbaryl (5·0)	30	14·7 ± 4·9§	27·7 ± 4·8¶	$P < 0.05$
	120	32·1 ± 13·4¶	77·0 ± 21·8¶	$P < 0.01$

* SKF525-A (25 mg/kg) given 30 min prior to saline or drug; N = 10 rats.
† Level of significance for treatment, alone and with SKF525-A.
‡ Average of 10 control days.
§ $P < 0.05$ compared to controls at same time after injection.
¶ $P < 0.01$ compared to controls at same time after injection.

the next treatment day (3–4 days later), they were given the same dose of the agent preceded by SKF525-A. This schedule was repeated at a later date. No difference in response to the same treatment was observed at any time, and the results reported are those obtained in the first treatment schedule. All behavioral data are given in Table 1 and all brain cholinesterase studies are reported in Table 2. The influence of SKF525-A

* Kindly supplied by Smith Kline & French Labs., Philadelphia, Pa.
† Kindly supplied by Union Carbide Corp., Chemical Division, New York, N.Y.

on the inhibition of avoidance behavior and brain enzyme at the time of peak action is graphically represented in Fig. 1.

The dose of eserine administered in these studies was without effect on discrete avoidance behavior. Pretreatment with SKF525-A resulted in a four-fold increase in shocks taken during the first 30 min after eserine, while nearly eight times as many

TABLE 2. INFLUENCE OF SKF525-A ON THE BRAIN ANTICHOLINESTERASE ACTIVITY OF ESERINE, COMPOUND 10854, AND CARBARYL*

Treatment (dose, mg/kg)	Min after inj.	Alone		With SKF525-A		Difference†
		No. of rats	Inhibition from controls (% ± S.E.)	No. of rats	Inhibition from controls (% ± S.E.)	
Eserine (0·16)	30	11	22·0 ± 5·5‡	11	59·5 ± 1·6‡	$P < 0.01$
	120	8	6·6 ± 2·7	6	32·6 ± 5·5‡	$P < 0.01$
Compound 10854 (0·5)	30	10	30·8 ± 1·8‡	6	54·8 ± 5·3‡	$P < 0.01$
	120	6	2·8 ± 1·7	6	14·6 ± 5·2‡	$P < 0.05$
Carbaryl (5·0)	30	10	35·8 ± 3·8‡	10	37·3 ± 6·7‡	Not signif.
	120	10	10·8 ± 2·2‡	10	13·6 ± 2·0‡	Not signif.

* SKF525-A (25 mg/kg) given 30 min prior to treatment; this was without effect by itself in 6 rats each studied at 60 and 150 min.

† Level of significance for treatment, alone and with SKF525-A.

‡ $P < 0.01$ compared to controls at same time after injection.

shocks were recorded during a 2-hr experimental session. With sacrifice times of 30 and 120 min, studies of brain cholinesterase inhibition after eserine (0·16 mg/kg) revealed 22% and 7% inhibitions. When the microsomal enzyme inhibitor was given, eserine showed 60% and 33% inhibitions, respectively, at those times.

Results obtained with compound 10854 during the first 30-min period revealed approximately twice as many shocks and almost twice as much cholinesterase inhibition when SKF525-A was given prior to the carbamate. Although differences exist at the 2-hr period for both parameters, with and without SKF525-A, these differences are not nearly so great as those obtained in the eserine studies.

The potentiation by SKF525-A of behavioral effects of carbaryl was similar to its potentiation with compound 10854. However, there was no concomitant enhancement of the anticholinesterase effects of carbaryl. No difference in the degree of brain enzyme inhibition was obtained in animals treated with SKF525-A and carbaryl from those given only carbaryl.

DISCUSSION

Results obtained in these studies clearly indicate that SKF525-A causes an enhancement of the behavioral effects induced by the carbamates studied. In addition, the anticholinesterase activity of eserine and compound 10854, but not of carbaryl, was similarly affected. All three agents are active cholinesterase inhibitors *in vitro* and thus do not require biological activation as do some phosphate enzyme inhibitors; additionally, their behavioral effects are antagonized by atropine, suggesting either

central anticholinesterase activity and/or direct cholinergic stimulation.* Since inhibition of liver microsomal enzymes results in a somewhat similar degree of enhancement of inhibition of behavioral and cholinesterase effects produced by eserine and compound 10854, it would appear that SKF525-A pretreatment allows a more persistent anticholinesterase effect, thus accounting for the potentiation of behavioral disruption.

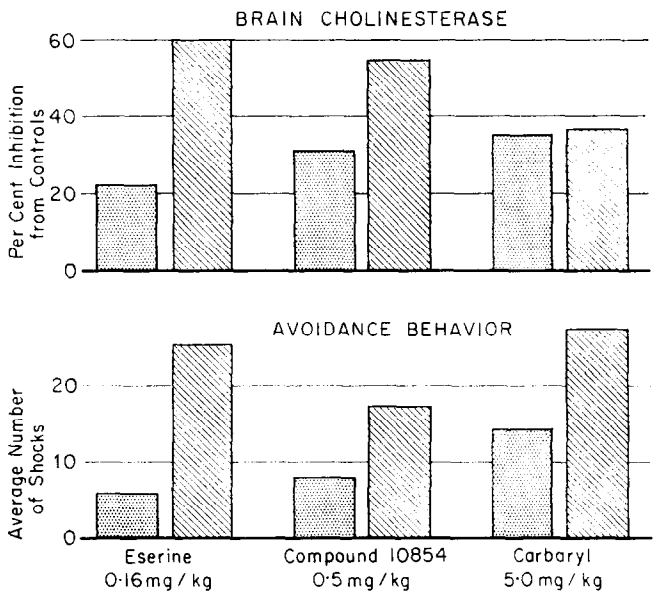

FIG. 1. Effect of SKF525-A on the inhibition of avoidance behavior and brain cholinesterase produced by 3 carbamates at the time of peak action; 30 min after carbamate injection for brain cholinesterase and cumulative 30 min for avoidance behavior. Dotted bars indicate effect of treatment alone. Hatched bars indicate effect of treatment when preceded by 25 mg SKF525-A/kg (30 min prior to carbamate).

Whether the presence of the naphtyhl moiety in carbaryl would be involved in the differential effects of SKF525-A is not at present known. It has been reported that about one third of an ingested dose of carbaryl is excreted as 1-naphthol, almost completely in the conjugated form;[11] this value is compatible with recoverable excretory quantities when 1-naphthol is given alone.[12] Dorough *et al.*[13] recently reported that a nonhydrolytic pathway for this agent may be an important step in its biological degradation. The authors reported that when carbaryl was incubated with fortified rat liver microsomes at least 5 carbamate metabolites involving modification of the methyl group or the ring were found. Two of these, notably the 4- and 5-hydroxy derivatives of carbaryl, were suggested as possible, important metabolites. Recent studies by Casida *et al.*† revealed that these metabolites may be formed only in small amounts, possibly not enough to account for the biological effects observed.

* Unpublished results from this laboratory.

† Personal communication.

Based upon these observations, it is suggested that carbaryl is converted to a metabolite which is devoid of cholinesterase inhibitory effects, is antagonized by atropine, and undergoes further metabolic alteration by liver microsomal enzymes. Inhibition of these enzymes by SKF525-A allows the accumulation of the metabolite, which yields an enhancement of behavioral effects but in no way influences, the degree of cholinesterase inhibition induced by the parent compound. Detailed information concerning the entire metabolic effects of carbaryl is obviously needed.

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