Effect of β-Diethylaminoethyl Diphenyl-*n*-propyl Acetate (SKF 525-A) on the Plasma Concentration of C^{14}-Labeled Neuromuscular Blockers

By FLOYD R. DOMER† and W. PINTO-SCOGNAMIGLIO

Although pretreatment with SKF 525-A potentiates the intensity and duration of neuromuscular blockade, it did not alter significantly the plasma radioactivity of dogs given C^{14}-labeled decamethonium (C10) or bis (dimethylaminobutoxy) 1,4-benzene diiodomethylate (1815, I.S.) or rabbits given 1815 I.S.

AMONG THE many biological actions of β-diethylaminoethyl diphenyl-*n*-propyl acetate (SKF 525-A) (1, 2) that of affecting neuromuscular transmission and muscular contraction was first reported by Navis, *et al.* (3). SKF 525-A was shown to potentiate greatly the blockade caused by a number of compounds possessing neuromuscular activity (4). This latter group of workers proposed the theory that the SKF 525-A caused its potentiation by displacing the neuromuscular blocking agent from nonspecific receptors to the neuromuscular junction. Subsequent work *in vitro* with a nitrocellulose membrane indicated that SKF 525-A indeed is quite potent in its ability to displace a neuromuscular blocker (5).

The present report deals with another attempt to elucidate the mechanism of action of SKF 525-A in potentiating neuromuscular blockade. The approach was that of using a C^{14}-labeled neuromuscular blocking agent and measuring the radioactivity in the blood of dogs and rabbits.

C^{14}-Labeled decamethonium (C10), 0.2 mg./Kg., or bis(dimethylaminobutoxy) 1,4-benzene diiodomethylate (1815, I.S.), 0.2 mg./Kg., were administered to dogs anesthetized with chloralose as previously described (4). Blood samples were taken from the femoral vein at 1, 2, 4, 7, 10, 15, and 30 minutes after the $C^{14}C10$ administration. At 45 minutes a second dose of the $C^{14}C10$ was given. Plasma samples were prepared and counted in a refrigerated liquid scintillation counter as previously described (6). A 10 mg./Kg. dose of SKF 525-A was given intravenously 10 minutes before the first dose of radioactive C10 or 1815, I.S. in the test experiments. Table I gives the results obtained in a pair of dog experiments with the SKF 525-A pretreatment. The circulating blood level of the neuromuscular blocking agent was higher than in the control animal during the first 4 minutes. Subsequently, it decreased more rapidly than in the control. Similar results were obtained in dogs given C^{14}-labeled 1815, I.S.

Rabbits were anesthetized with urethane (1.5 Gm./Kg. s.c.), given artificial respiration throughout the experiment, and C^{14} 1815, I.S. (0.25 mg./Kg.) was given intravenously. Blood samples were obtained from the heart at 1, 5, and 10 minutes. At 20 minutes, a second dose of the C^{14} 1815, I.S. was given and a second series of blood samples taken at 1, 5, and 10 minutes. The test animals were given SKF 525-A (10 mg./Kg. i.v.) 10 minutes prior to the first administration of C^{14} 1815, I.S. Table II gives the results obtained in five rabbit experiments. There was no significant difference in the circulating blood level of radioactive neuromuscular blocker between control animals and those pretreated with SKF 525-A ($p > 0.2$). However, the treated animals did have slower rates of decrease in blood radioactivity.

The fact that there was no elevation in the circulating blood level of C^{14}-labeled neuromuscular blocking agents in the rabbit or the dog would at first appear to be contrary to the hypothesis of displacement from nonspecific receptors to the neuromuscular junction. However, this need not necessarily be the case. Waser (7) using C^{14}-labeled calabash curarine found that pretreatment with SKF 525-A doubled the amount of radioactivity found in the end-plates of mice diaphragms. This indicates directly that there is an increased interaction between the labeled compound and the endplate in the pretreated animals. Similarly, in our experiments, the SKF 525-A pretreatment could have increased the affinity of the receptors for the labeled compound without affecting the total number of receptors involved. This would result in a decrease in the amount of circulating radioactive compound. Another possibility could be that the neuromuscular receptors represent such a small percentage of all the body receptors that the change caused by the SKF 525-A would be too small to be detected by our procedure. Finally, the theory advanced by Paton (8) that the *rate* of attachment

TABLE I.—PLASMA LEVELS OF C^{14}-LABELED DECAMETHONIUM IN DOGS

Injection	Time, min.	—$C^{14}C10$, mcg./ml.— Control[a]	Pretreated with SKF 525-A[a]
SKF 525-A, 10 mg./Kg. i.v.	−10	...	...
$C^{14}C10$, 0.2 mg./Kg. i.v.	0	...	...
	1	3.05	3.53
	2	1.61	2.71
	4	1.28	1.59
	7	1.93	1.39
	10	1.78	1.08
	15	1.63	0.76
	30	1.37	0.44
$C^{14}C10$, 0.2 mg./Kg. i.v.	45	...	...
	46	2.95	3.85
	47	2.39	3.08
	49	2.02	2.06
	52	1.17	1.74
	55	1.24	1.32
	60	1.32	1.06
	75	1.18	0.67

[a] Each column represents the results obtained in a single dog experiment.

Received March 6, 1963, from the Istituto Superiore di Sanita, Rome, Italy.
Accepted for publication June 5, 1963.
This research has been sponsored in part by Contract No. AF61(052)-469 from the Research Division, Air Research and Development Command, U. S. Department of the Air Force, through its European office.

† Present address: Department of Pharmacology, Tulane University, New Orleans, La.

TABLE II.—PLASMA LEVELS OF C^{14}-LABELED 1815, I.S. IN RABBITS

Injection	Time, min.	C^{14} 1815, I.S., mcg./ml. Control Mean ± S.E.[a]	C^{14} 1815, I.S., mcg./ml. Pretreated with SKF 525-A Mean ± S.E.[a]
SKF 525-A, 10 mg./Kg. i.v.	−10	...	...
	0	...	...
C^{14} 1815, I.S. 0.25 mg./Kg. i.v.			
	1	3.22 ± 0.73	2.71 ± 0.70
	5	1.48 ± 0.29	1.52 ± 0.66
	10	0.94 ± 0.34	1.26 ± 0.45
	20	...	...
C^{14} 1815, I.S. 0.25 mg./Kg. i.v.			
	21	3.44 ± 1.57	4.40 ± 1.41
	25	2.04 ± 0.99	3.01 ± 1.58
	30	1.78 ± 1.01	2.76 ± 1.80

[a] Five rabbits used in each case.

to, and detachment from, receptors is important for stimulation would also be compatible with the present findings. This would mean that the SKF 525-A is increasing the rate at which the neuromuscular blocker-receptor combination is being made and broken without having influence on the total amount of drug being present.

Evidence that SKF 525-A itself has an effect upon neuromuscular transmission and receptor excitability have been found by a number of authors (3, 9, 10). This makes it quite difficult to separate clearly the action(s) of SKF 525-A involved in the observed potentiation of neuromuscular blocking agents. Further attempts will be necessary to clarify this problem.

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Synthesis of N-Substituted and N,N′-Disubstituted Benzyl Derivatives of 5,5-Disubstituted Barbiturates

By EDWARD J. ROWE and MELVIN H. WEINSWIG

N-Substituted and N,N′-disubstituted benzyl derivatives of barbital, phenobarbital, and amobarbital have been synthesized by the use of a strongly basic anion-exchange resin. The barbiturate is first absorbed on the resin and the resin then agitated mechanically with an ethanol solution of benzyl chloride. The reaction proceeds at room temperature. The yields in all cases ranged from 50 to 80 per cent.

IN A PREVIOUS REPORT (1) a method was described for the synthesis of benzyl ethers of a variety of phenols by the use of a strongly basic anion-exchange resin. The method involved the reaction of the phenolate form of the resin with benzyl chloride. This report presents an analogous method for the synthesis of N-benzyl and N,N′-dibenzyl derivatives of three 5,5-disubstituted barbituric acids (phenobarbital, barbital, and amobarbital). The synthesis of these derivatives involves the reaction of the barbiturate form of a strongly basic anion-exchange resin with benzyl chloride. The resin employed is based on polystyrene and contains quaternary ammonium groups. The reaction proceeds at room temperature. A mixture of N-benzyl and N,N′-dibenzyl derivatives is obtained from the reaction. Separation of the derivatives is based upon the solubility of the N-benzyl derivative in dilute alkali. Table I summarizes the benzyl derivatives of phenobarbital (5-ethyl-5-phenyl barbituric acid), barbital (5,5-diethyl barbituric acid), and amobarbital (5-ethyl-5-isoamyl barbituric acid) prepared by the use of a strongly basic anion-exchange resin. The yields are based upon the amount of the barbiturate converted to the N-benzyl and N,N′-dibenzyl derivatives. The N,N′-dibenzyl derivative is the main product when an equivalency of the resin in the OH form is used with the barbiturate form and the quantity of the benzyl chloride is doubled.

Received June 3, 1963, from the College of Pharmacy, Butler University, Indianapolis, Ind.
Accepted for publication June 27, 1963.

EXPERIMENTAL

Resin Preparation.—Dowex 1X4[1] supplied commercially in the chloride form (20–50 mesh) was converted to its hydroxyl form by the usual column

[1] Manufactured by Dow Chemicel Co., Midland, Mich., and purchased from J. T. Baker Co., Phillipsburg, N. J.