Effects of β-Diethylaminoethyl Diphenylpropylacetate Hydrochloride on Three Convulsants and on Propallylonal

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β-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A) increased the LD_{50}s of nikethamide, pentylenetetrazol, and picrotoxin in albino mice when given intraperitoneally 1 hour earlier. The maximum action of picrotoxin in mice subcutaneously pretreated with SKF 525-A developed in about 4 hours; action was still marked at 8, but largely absent at 16 hours. SKF 525-A induced full effect when given intraperitoneally immediately before picrotoxin. It also markedly increased the potency of picrotoxin in normal and in barbitalized rats, using a multiple injection technique. However, SKF 525-A did not influence the toxicity of pentylenetetrazol in normal or barbitalized rats employing this technique. Barbital greatly increased the LD_{50}s of pentylenetetrazol and picrotoxin in rats, as shown by the multiple injection method. SKF 525-A markedly prolonged the hypnotic effect of propallylonal without increasing the number of delayed deaths. Its use is recommended for biotransformation studies of the four substances used.

BETA-diethylaminoethyl diphenylpropylacetate hydrochloride, hereafter referred to as compound SKF 525-A, has been shown to potentiate or prolong the action of a great variety of drugs. Cook, *et al.* (1, 2), in mice and rats, demonstrated that it prolonged the action of secobarbital, pentobarbital, hexobarbital, amobarbital, butethal, hexothal, phenobarbital, and chloral hydrate; however, barbital and thiopental depression in mice was not altered. On the other hand, Achor and Geiling (3), administering SKF 525-A intraperitoneally rather than orally, obtained prolongation of thiopental hypnotic action using similar and higher dosage of SKF 525-A. Macko, *et al.* (4), reported that this compound increased the central nervous system stimulation of *d*-amphetamine and strychnine and greatly increased the toxicity of the latter. Loewe (5) noted that the CNS-stimulating properties of yohimbine upon the butallylonal-yohimbine ejaculatory effect in mice was augmented by SKF 525-A. Axelrod and co-workers (6) and Cooper, *et al.* (7), reported that much of the effect of SKF 525-A is due to its ability to inhibit the biotransformation of the drugs in question. It becomes widely localized in rat organ tissues, especially fat, is present in the body after 24 hours, and is practically all metabolized *in vivo* (6). Swinyard and collaborators (8) have shown potentiation of the action of a series of antiepileptic drugs by SKF 525-A; however, the potentiation was not considered due entirely to inhibition of biotransformation of these drugs inasmuch as SKF 525-A possesses some anticonvulsant activity of its own in high dosage.

SKF 525-A is effective *in vivo* when administered up to 20 hours prior to the drugs whose effects are capable of being influenced; also, prolongation of hexobarbital depression could be demonstrated by administering SKF 525-A during hexobarbital hypnosis, but not after recovery (1, 2). It is equally potent when administered orally, intraperitoneally or intravenously (2). A suitable intraperitoneal and oral dose for mice and rats is given as 50 mg./Kg., and the optimum pretreatment time as 40 to 60 minutes prior to the administration of the drug under study (1).

The present series of experiments was designed to obtain information concerning the effects of SKF 525-A upon the median lethal doses of nikethamide, pentylenetetrazol, and picrotoxin in mice. Special attention was given to methods of administration, time interval for pretreatment, and duration of action with picrotoxin. Tests were also conducted in rats to note what influence barbital anesthesia might have upon the effect of SKF 525-A upon pentylenetetrazol and picrotoxin toxicity. Finally, the effect of SKF 525-A upon the duration of depression, incidence of delayed death, and sex variation with propallylonal in rats was studied.

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EXPERIMENTAL

General.—Male mice of Swiss albino stock, weighing 16–26 Gm. and randomized according to weight within each suitably sized group, were used in the niketharnide and picrotoxin experiments. With pentylenetetrazol, six males and six females were used in each group. All mice were kept for at least 1 week in temperature-controlled animal quarters on a diet of Purina Laboratory Chow and water *ad libitum* until the time of experimentation. After injection the mice were kept in individual large glass beakers and the room temperature maintained at 25 ± 0.5°. SKF 525-A was administered as a 1.0% solution in distilled water. A 3% solution of niketharnide, a 1% solution of pentylenetetrazol, or a 0.05% solution of picrotoxin was employed; distilled water served as the diluent. Suitable logarithmic dosages were utilized and the median convulsive and lethal doses (CD_{50}s and LD_{50}s) were determined by the Reed-Muench and Pizzi methods, using logarithms (10, 11). An equivalent volume of 0.9% sodium chloride solution was injected into the control groups. Whenever an interval of 3 hours or longer occurred between the injection of SKF 525-A and the picrotoxin, the mice were returned to the stock cages immediately after the administration of SKF 525-A and allowed food and water until 1 hour prior to the scheduled administration of the pictrotoxin. They were then reweighed, placed in individual containers, and the picrotoxin dose calculated from the final weights. In a few cases, when a very large number of mice were under study, it was impractical to complete a series in one day so the series was continued on a later day. All intraperitoneal injections were made in the upper left quadrant of the abdomen, while all subcutaneous injections were made under the loose skin of the lower back.

In the further studies of the effects of SKF 525-A upon pentylenetetrazol and picrotoxin, Holtzman male rats in groups of 10 were employed to determine the CD_{50}s and LD_{50}s in normal and barbitalized animals. Each analeptic was injected subcutaneously every 10 minutes, using ten different sites in rotation according to the technique previously developed by Holck (12). In case of pentylenetetrazol, 200 mg./Kg. of barbital sodium (10% solution) or 2 ml./Kg. of saline were injected intramuscularly 10 minutes prior to injecting 50 mg./Kg. (5% solution) of SKF 525-A or 1 ml./Kg. of saline intraperitoneally. Sixty minutes later an initial dose of 9 mg./Kg. (0.9% solution) of pentylenetetrazol was administered to the nonbarbitalized rats and 25 mg./Kg. (5% solution) to the barbitalized rats and their controls. Barbital was chosen as the hypnotic agent because its action is not prolonged by pretreatment with SKF 525-A (2); it also has more recently been shown that SKF 525-A does not alter its induction time (13). The age of the first two groups of rats was about 5 months, and of the last three groups about 10 months.

In the picrotoxin tests, the barbital sodium dose was 225 mg./Kg. (11.25% solution); the dose of picrotoxin per injection was 0.7 mg./Kg. (0.07% solution) for nonbarbitalized rats and 1 mg./Kg. (0.1% solution) for barbitalized rats. It was necessary to increase the dose of picrotoxin because tolerance developed to the lower dose. The ages of the animals in the two sets of tests were 3 and nearly 5 months, respectively. All rats were kept under conditions similar to those previously described for the mice.

In the studies with propallylonal, this drug was converted to its sodium salt by the addition of an appropriate amount of 0.2 *N* sodium hydroxide solution and diluted to the desired volume with freshly boiled, triple distilled water. Holtzman rats of both sexes were used, because female rats in previous studies usually have proven more sensitive to propallylonal than the males. Rats, 4–6 months old, were used in the first experiment in which 30 mg./Kg. (3% solution) of propallylonal were injected subcutaneously as a single dose. In the experiment using a 50 mg./Kg. dose, a 4% solution was employed and injected intraperitoneally; these rats were 4 months old. In both tests the rats were fasted overnight but allowed water. Intraperitoneal administration of 50 mg./Kg. (2.5% solution in the first, 5% in the second test) of SKF 525-A, or of an equal volume of saline, was made 1 hour prior to the barbiturate medication. The animals were observed during 6 days following propallylonal administration for incidence of delayed death.

RESULTS

Concomitant with administering the analeptics to mice it was ascertained that the stock used had a resistance to SKF 525-A quite similar to that for intraperitoneal administration reported by Cook, *et al.* (1). Thus, with a 1% solution, a temperature of 25° and 10 male mice per group, none died with 100, 5 with 141, and all with 200 mg./Kg. However, with subcutaneous injection the resistance was much higher in that 10 mice survived 100, 200, and 400 mg./Kg., respectively; a 2% solution was used. The results of the experiments with the analeptics in mice and rats are presented in Tables I and II.

Following subcutaneous administration of 30 mg./Kg. of propallylonal, only $^3/_{24}$ rats pretreated with SKF 525-A lost the righting reflex compared with none of the controls. No delayed deaths occurred in either group. With 50 mg./Kg. of propallylonal given intraperitoneally, the righting reflex was recovered in 5 control females in 186 ± 36 and in 5 pretreated ones in 345 ± 15 minutes; $P < 0.01$. In the males the minutes were 73 ± 6.3 for 4 out of 5 controls and 238 ± 24 for 5 pretreated animals; $P < 0.01$. P was about 0.03 for the sex variation in the controls and 0.02 in the pretreated rats. Although the depression time was significantly prolonged by SKF 525-A, the number of delayed deaths was not increased, being $^2/_5$ in the control and pretreated females, respectively, and $^1/_5$ in similar male groups.

DISCUSSION

An outstanding finding of our studies upon albino mice was that the ratio of the LD_{50} of the controls over that of those pretreated with SKF 525-A was smaller for pentylenetetrazol and for niketharnide than for picrotoxin. It was 1.24 for pentylenetetrazol and 1.32 for niketharnide, as compared with 2.63 and 2.81, respectively, in two tests with picrotoxin. The last ratios are in good agreement with the 2.6 reported by Macko, *et al.* (4), for strychnine.

TABLE I.—EFFECT OF β-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE HYDROCHLORIDE (SKF 525-A) ON THE INCIDENCE OF ACUTE DEATH WITH PENTYLENETETRAZOL, NIKETHAMIDE, AND PICROTOXIN IN ADULT ALBINO MICE

	SKF 525-A, mg./Kg.	Route	Hours after SKF 525-A	No. of Mice/ Mice per Group[a]	LD_{50} ± S.E., mg./Kg.	*P*	
				Pentylenetetrazol			
A	Control	i.p.	1	60/12	77.5 ± 3.4	...	
B	50	i.p.	1	72/12	62.1 ± 2.7	<0.005	
				Nikethamide			
A	Control	i.p.	1	40/10	325 ± 19	...	
B	25	i.p.	1	40/10	283 ± 17	>0.1	A *vs.* B
C	Control	i.p.	1	50/10	327 ± 22	...	
D	50	i.p.	1	40/10	248 ± 15	<0.02	C *vs.* D
				Picrotoxin			
A	Control	i.p.	1	52/13	6.24 ± 0.25	...	
B	25	i.p.	1	50/10	2.54 ± 0.19	<0.001	A *vs.* B
C	Control	i.p.	1	60/10	6.54 ± 0.38	...	
D	50	i.p.	1	60/10	2.47 ± 0.16	<0.001	C *vs.* D
E	Control	i.p.	1	50/10	2.70 ± 0.21[b]	...	
F	50	i.p.	1	70/10	0.84 ± 0.07[b]	<0.001	E *vs.* F
A	50	s.c.	1	40/10	4.42 ± 0.32	...	
B	50	s.c.	2	40/10	3.53 ± 0.27	<0.07	A *vs.* B
C	50	s.c.	4	50/10	3.17 ± 0.20	<0.01	A *vs.* C
D	50	s.c.	8	50/10	4.05 ± 0.23	<0.02	C *vs.* D
E	50	s.c.	16	30/10	5.82 ± 0.30	<0.001	D *vs.* E
A	Control	i.p.	1	36/12	7.13 ± 0.38	...	
B	50	i.p.	3	48/12	3.31 ± 0.17	<0.001	A *vs.* B
C	50	s.c.	3	60/12	3.54 ± 0.17	<0.001	A *vs.* C
D	50	s.c.	6	60/12	3.75 ± 0.21	<0.001	A *vs.* D
A	Control	i.p.	0	45/15	5.96 ± 0.26	...	
B	50	i.p.	0	60/15	2.23 ± 0.10	<0.001	A *vs.* B
C	50	i.p.	1	60/15	2.10 ± 0.10	<0.001	A *vs.* C

[a] Numbers of mice in groups not showing either 100% negative or 100% positive responses/number of mice per dosage group. Represent CD_{50}s.

TABLE II.—EFFECT OF β-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE HYDROCHLORIDE (SKF 525-A) ON THE INCIDENCE OF CONVULSIONS, DEVELOPMENT OF TOLERANCE, AND LETHAL DOSE WITH PENTYLENETETRAZOL AND PICROTOXIN, USING THE MULTIPLE INJECTION METHOD IN NORMAL AND BARBITALIZED ADULT MALE ALBINO RATS

Injection, mg./Kg.	Barbital Sodium, mg./Kg.	SKF 525-A, mg./Kg.	CD_{50} ± S.E., mg./Kg.	LD_{50} ± S.E., mg./Kg.	*P*	
			Pentylenetetrazol			
9	None	None	...	134 ± 5.9[a]	...	
9	None	50	...	119 ± 8.5[a]	>0.1	
25	None	None	75 ± 6.5	130 ± 19.6[a]	...	
25–50[b]	200	None	...	1085	...	
25–50[b]	200	50	...	1105	...	
			Picrotoxin			
0.7	None	None	4.97 ± 0.49	9.17 ± 0.32	<0.001	(CD_{50}s)
0.7	None	50	3.15 ± 0.12	5.46 ± 0.17	<0.001	(LD_{50}s)
1.0	225	None	[c]	[c]	...	
1.0	225	50	7.8 ± 0.13	12.4 ± 0.22	...	

[a] There was evidence of development of tolerance in a few of the rats. [b] No convulsions occurred in either group after 12 injections (300 mg./Kg.), hence the dose per injection was raised to 50 mg./Kg. [c] Only one rat convulsed and none died after 20 injections (20 mg./Kg.). All 10 rats made uneventful recoveries and were alive 5 days later. The average doses producing recovery of the righting reflex were 6.0 ± 0.45 mg./Kg. for the controls and 6.5 ± 0.27 mg./Kg. for the SKF 525-A premedicated rats; $P < 0.3$.

The results with picrotoxin agree with those of Cook, *et al.* (1), in that the action occurred almost immediately after intraperitoneal administration of SKF 525-A and was fully developed in 1 hour; it lasted more than 16 hours after subcutaneous administration. Although marked action was noted after 1 hour, maximum action with subcutaneous pretreatment with SKF 525-A did not occur until several hours later, indicating a slower absorption of SKF 525-A after this method of injection. The much more gradual absorption is also indicated by the mentioned much higher fatal dose of SKF 525-A with subcutaneous compared with intraperitoneal administration. The results with albino rats also agree in principle with those of Cook, *et al.* (2), in that the increase in duration of hypnotic action after propallylonal occurred without increasing the number of delayed deaths; however, the prolongation of depression was much less marked than in case of hexobarbital. Tolerance to pentylenetet-

razol occurred with and without SKF 525-A pretreatment in all of the barbitalized rats and in some of the normals, using a multiple injection technique. Cook, *et al.* (9), have reported a similar absence of interference with development of tolerance to morphine by SKF 525-A; it did not change its LD_{50}.

On the other hand, pretreatment with SKF 525-A markedly lowered the fatal dose of picrotoxin in barbitalized rats and significantly decreased the convulsive and fatal doses in normal rats. The results with barbitalized rats, pretreated with SKF 525-A, were unusually uniform in that the standard errors were less than 1.8% of the CD_{50}s and LD_{50}s of picrotoxin, compared with 3.5% and 3.1% in normal, pretreated rats.

The major factor of SKF 525-A effect is due to the inhibition of biotransformation of substances susceptible to its influence. The extensive review by Brodie (14) involving, in part, numerous *in vivo* and *in vitro* studies by his own staff, indicates that a similar inhibitory effect by SKF 525-A may be exerted on a variety of metabolic pathways or, as stated by Cooper, *et al.* (7), it inhibits a factor or factors possessed in common by a diversity of drug enzyme systems. Depression of bioacetylation and of acetylcholinesterase activity has since been reported by Kuriaki and Marumo (15). The mechanism involved in the case of the three analeptic drugs tested by us remains to be determined. With propallylonal, SKF 525-A may prevent the replacement of Br by an OH group and subsequent molecular rearrangement of the ketone form to give an acetonyl group, which leads to formation of a barbiturate that is much weaker and slower in action than the β-bromallyl derivative (16, 17).

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Temperature-Induced Rheological Variability in an Emulsion System

By JAMES C. BOYLAN, H. GEORGE DeKAY, and GILBERT S. BANKER

A study has been made on the effect of emulsification temperature and cooling rate on certain physical properties of a beeswax-mineral oil emulsion. The emulsion, which was prepared at five temperatures and cooled at three rates, was evaluated for plastic viscosity, thixotropy, yield value, water separation rate, and initial average particle size. It was shown that both the emulsification temperature and cooling rate significantly altered these properties.

IN THE PAST few decades, emulsions have been studied from many viewpoints. Despite this, the formulation, preparation, and stability of most emulsion products remains, to a large extent, an empirical art. Investigations to date have failed to pinpoint the rheological characteristics of many emulsions.

It is generally agreed that an increase in temperature facilitates emulsification (1–3). One author (4) contends in rather vague and general terms that an emulsification temperature should be chosen which will give the optimum dispersion to the materials being emulsified. Spalton (5) specifies that a freshly prepared wax emulsion should be allowed to cool slowly since rapid cooling may result in a granular product. Wellman (6) found that the solubility of the soap, as controlled by temperature, is an important factor in emulsion formation. Several studies have been conducted attempting to relate viscosity of emulsions to temperature (7–9).

In the area of particle size and its effect on viscosity, Sherman (10) states that there is a frequent increase in viscosity when an emulsion is homogenized. He feels that this increase in viscosity is due to a decrease in particle size,

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