Inhibitory Actions of β-Diethylaminoethyl Diphenyl-n-Propyl Acetate (SKF 525-A) on Muscle Contraction. (26564)

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It has been known for several years that β-diethylaminoethyl diphenyl-n-propyl acetate (SKF 525-A) has an inhibitory action on the microsomal enzymes concerned with detoxification of foreign compounds. SKF 525-A has been shown to prolong the duration of action of a number of drugs which are detoxified by various diverse pathways; for example, by deamination, by N-demethylation, by side chain oxidation, by hydroxylation. These effects were reviewed by Brodie(1). SKF 525-A also inhibits some non-microsomal reactions including the formation of glucuronides and de-esterification of procaine. It has been shown to inhibit the soluble enzyme nitro-reductase(1).

The literature has been solely concerned with observations on the effects of SKF 525-A on enzyme systems; no work has been reported on the effects of SKF 525-A acting directly on skeletal and smooth muscle. The experiments to be described here are concerned with the effects of SKF 525-A on the isolated frog rectus abdominis muscle, the isolated rabbit ileum, and the frog gastrocnemius muscle.

Methods. (a) The rectus abdominis muscle of the frog (*Rana pipiens*) was suspended in an isolated organ bath containing frog Ringer's solution consisting of 111 mM NaCl, 1.9 mM KCl, 1.1 mM $CaCl_2$, 2.4 mM $NaHCO_3$.

(b) Segments of washed rabbit ileum were suspended in oxygenated Tyrode's solution containing 137 mM NaCl, 2.7 mM KCl, 0.90 mM $CaCl_2$, 1.06 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, 5.5 mM dextrose. The solution was kept at 37°C, and contractions of the muscle were recorded on a kymograph drum using an ink recorder.

(c) The femoral artery of a pithed frog was cannulated just below the bifurcation of the aorta with a polyethylene cannula. The sciatic nerve was exposed and shielded electrodes were attached to it. The nerve was stimulated with square wave impulses of 1 millisecond duration at a frequency of 2 per second. Silver wire electrodes were embedded at each end of the gastrocnemius muscle, and the muscle was stimulated with square wave impulses of 20 milliseconds duration at a frequency of 2 per second. The threshold voltage which caused muscle contraction was measured for both nervous and muscle stimulation. Drugs were introduced via the polyethylene cannula.

(d) The effect of SKF 525-A on cholinesterase activity was measured as follows: 5 ml of human blood plasma were diluted with 5 ml sodium barbital solution (0.04 M) adjusted to pH 9.0. Ten ml of a 1% acetylcholine iodide solution were added and the volume made up to 50 ml with distilled water or with the solution under test. Changes in pH were measured at 1 minute intervals using a glass electrode.

Results. (a) *Frog rectus abdominis muscle.* SKF 525-A in a concentration of 500 μg/ml caused a slow steady contraction of the muscle which took 5 minutes to reach its peak. After this treatment the muscle was completely unresponsive to acetylcholine (ACh), and ACh response could not be restored even after repeated washings with fresh Ringer's solution (Fig. 1). Doses of 10 μg ACh/ml were given and the height of contraction observed in the presence of different concentrations of SKF 525-A. 0.1 μg/ml had no effect whereas 1.0 μg caused a 30% inhibition, and 100 μg caused complete inhibition of ACh-induced contractions.

The inhibition of the ACh effect by SKF 525-A was compared with that produced by d-tubocurarine. When SKF 525-A in a concentration of 10 μg/ml was added at the height of an ACh-induced contraction, no

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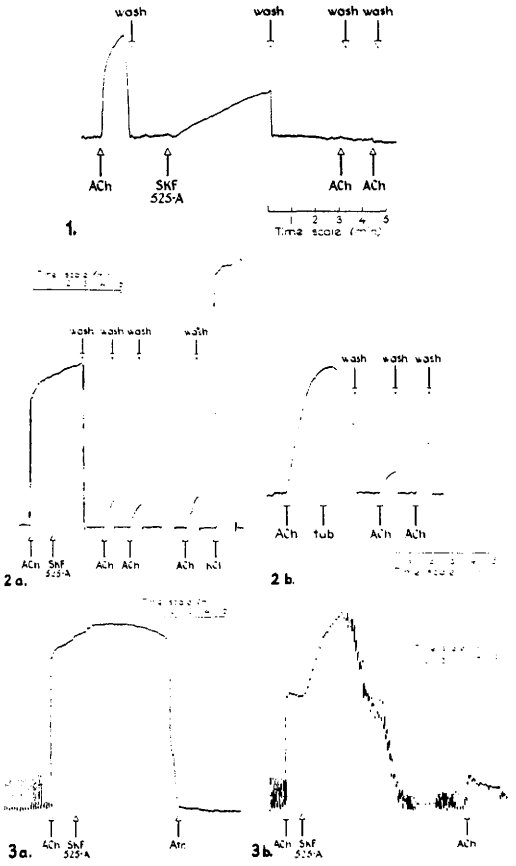

FIG. 1. Isotonic contractions of isolated frog rectus abdominis muscle in presence of ACh (10 μg/ml) and of SKF 525-A (500 μg/ml).

FIG. 2a. Isotonic contractions of isolated frog rectus abdominis muscle in presence of ACh (10 μg/ml), SKF 525-A (10 μg/ml), and potassium chloride (2%). 2b. Same after ACh (10 μg/ml) and d-tubocurarine (10 μg/ml).

FIG. 3a. Contractions of isolated rabbit ileum in presence of ACh (0.3 μg/ml), SKF 525-A (0.15 μg/ml), and atropine sulfate (0.15 μg/ml). 3b. Same after ACh (0.3 μg/ml) and SKF 525-A (1.5 μg/ml).

immediate relaxation occurred as was found with the same dose of d-tubocurarine (Fig. 2a, b). It was also observed that a muscle previously treated with SKF 525-A (10 μg/ml) was still refractory to ACh after being suspended in an eserine solution (5 μg/ml) for 20 minutes, whereas a muscle previously treated with d-tubocurarine and then suspended in the eserine solution responded to ACh after 20 minutes. After complete inhibition with SKF 525-A the muscle still contracted maximally in presence of a 2% KCl solution (Fig. 2a).

Procaine, a compound with a structure similar to SKF 525-A, was tested on the muscle preparation and found to have no effect on its response to ACh. The effect of atropine sulphate was studied. A concentration of 13 μg/ml produced rhythmic contractions of the muscle with increasing tension. After rinsing with fresh Ringer's solution the muscle was insensitive to ACh (10 μg/ml). In low doses atropine sulphate neither caused rhythmic contractions nor inhibited the ACh response.

(b) *Rabbit ileum.* 0.15 μg/ml SKF 525-A caused initially a slight potentiation of a contraction induced by ACh. On the other hand atropine in a similar concentration caused relaxation of the ileum and also stopped the peristaltic waves. A larger dose of SKF 525-A (1.5 μg/ml) caused a marked potentiation of the ACh contraction followed by relaxation. The muscle though still exhibiting spontaneous movement was now almost insensitive to ACh (Fig. 3a, b).

(c) *Frog gastrocnemius muscle.* When SKF 525-A (100 μg/ml) was injected in 0.2 ml doses into the femoral artery, the threshold to sciatic nervous stimulation rose. However, further experiments indicated that the effect was seen not only when the nerve was stimulated but also when the muscle was stimulated directly. Subsequently it was found that if the response to nervous stimulation was abolished by d-tubocurarine, which left the threshold to muscle stimulation unaltered, then addition of SKF 525-A abolished the response to muscle stimulation (Table I).

(d) *Cholinesterase.* SKF 525-A in a concentration of 2 μg/ml caused a 67% inhibition of plasma cholinesterase. In comparison, eserine in a concentration of 1 μg/ml caused complete inhibition.

Discussion. The observations reported here indicate that SKF 525-A has actions in addition to those previously described. SKF 525-A has been regarded as a drug of very low toxicity; the LD_{50} for rats has been reported as 2140 ± 400 mg/kg(2) and for mice approximately 540 mg/kg(3). All the reported actions of SKF 525-A have been concerned with detoxifying enzymes and its effect on them. In such *in vivo* experiments no side effects have been reported.

TABLE I. Effect of Saline (0.9% NaCl), d-Tubocurarine (1 μg/ml), and SKF 525-A (100 μg/ml) on Electrical Stimulation of Frog Gastrocnemius Muscle.

Time (min.)	Solution	Threshold for Nervous stimulation (volts)	Threshold for Muscle stimulation (volts)
0	.1 ml saline	.065	.32
5	*Idem*	.072	.27
10	"	.070	.29
15	"	.071	.26
20	.1 ml d-tubocurarine	.076	.27
25	*Idem*	.080	.30
30	"	.085	.25
35	"	.110	.27
40	"	34	.28
45	"	>100	.25
50	.1 ml d-tubocurarine + .1 ml SKF 525-A	"	.34
55	*Idem*	"	.38
60	"	"	.50
65	"	"	10
70	"	"	>100
75	"	"	"
80	"	"	"
85	"	"	"
90	"	"	"

The mechanism of the actions of SKF 525-A reported here is difficult to conceive. The most striking and consistent effect was an inhibition of muscle responses to acetylcholine. On the frog rectus muscle SKF 525-A would seem to act by combining with some ACh-sensitive receptor. The initial increase in tension could then be attributed to activation of the receptor site as the drug combined with it. This is similar to the explanation for the acetylcholine-like effect of atropine on the Mytilus gill cilia put forward by Bülbring, Burn, and Shelley(4). The binding appeared to be of a very tenacious nature since the muscle remained insensitive to ACh even after repeated washings with frog Ringer's solution.

The action of SKF 525-A on the rabbit ileum was unusual in that spontaneous rhythmic activity remained after addition of the drug, although the preparation was now insensitive to ACh. With atropine both the spontaneous waves and the response to ACh were abolished. The ileum differed from the frog rectus abdominis muscle in that inhibition of the ACh response could be reversed by frequent washings with fresh Tyrode's solution.

The experiments on stimulation of the frog gastrocnemius muscle indicated that SKF 525-A acted directly on the muscle and not exclusively on the myoneural junction. The frog gastrocnemius muscle was highly sensitive to SKF 525-A, and doses which completely inhibited the response to electrical stimulation had little effect on the responses to electrical stimulation of the other muscles of the same leg.

The inhibition of pseudocholinesterase may be of significance in that no physiological role has been assigned to this enzyme. Its concentration in rat liver is very high, and it may be that it is in some way linked with the microsomal enzymes. The non-specific effects of SKF 525-A might thus be mediated through inhibition of pseudocholinesterase. This would explain how SKF 525-A can inhibit so many diverse systems by a single mechanism. An interesting corollary to this suggestion is a recent observation (Milton, unpublished) that physostigmine a well known pseudocholinesterase inhibitor potentiates barbiturate sleeping time, a prominent property of SKF 525-A.

The significance of the results reported here is difficult to assess in absolute terms, but these data indicate that SKF 525-A exhibits pharmacological properties which are not related to its well known inhibition of detoxifying enzymes.

Summary. 1. SKF 525-A caused an increased tension in the isolated frog rectus abdominis muscle. After treatment with SKF 525-A the response to acetylcholine (ACh) was inhibited, and the inhibition could not be reversed by repeating washing of the muscle with fresh Ringer's solution. The inhibition differed from that observed with d-tubocurarine and atropine. 2. SKF 525-A had no direct effect in low concentrations on the isolated rabbit ileum, but it prevented spontaneous rhythmic activity in higher concentrations. It inhibited the effect of ACh on the gut and this inhibition was reversed by repeated washings with fresh Tyrode's solution. 3. SKF 525-A raised the threshold to direct electrical stimulation of the frog gastrocnemius muscle. 4. SKF 525-A inhibited the enzyme pseudocholinesterase. 5. These data are not fully explained, but the results indi-

cate that SKF 525-A has properties which differ markedly from those previously reported concerning the inhibition of microsomal detoxifying enzymes.

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1. Brodie, B. B., *J. Pharm. & Pharmacol.*, 1956, v8, 1.

2. Cook, L., Toner, J. J., Fellows, E. J., *Fed. Proc.*, 1953, v12, 313.

3. Toner, J. J., Cook, L., Fellows, E. J., *ibid.*, 1953, v12, 373.

4. Bülbring, E., Burn, J. H., Shelley, H. J., *Proc. Roy. Soc.* (London) B, 1953, v141, 445.

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Effect of Nicotinic Acid and Related Compounds on Incorporation of Mevalonic Acid into Cholesterol. (26565)

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The hypocholesterolemic effect of nicotinic acid administered orally to humans was first reported by Altschul *et al.*(1). Their results were confirmed by others(2,3). Nicotinic acid was later shown to lower serum cholesterol levels in cholesterol-fed rabbits(4), and a similar effect was observed in dogs(5). The mechanism by which nicotinic acid exerts the hypocholesterolemic effect has not been elucidated. Merrill(6) and later Hardy *et al.*(7) reported an increased incorporation of acetate-1-C^{14} into liver sterols in the presence of nicotinic acid, both *in vitro* and *in vivo*. However, Perry(8) found a decreased incorporation of acetate-2-C^{14} into cholesterol in rat liver slices incubated with nicotinic acid. Kritchevsky *et al.*(11) showed that nicotinic acid-treated rat liver mitochondrial preparations oxidize cholesterol to a greater extent than do similar preparations from control rats.

In the present experiments the effect of nicotinic acid and related compounds on incorporation of mevalonic acid-2-C^{14} (MVA-2-C^{14}) into non-saponifiable material (NSF, largely sterols) by rat liver homogenates was investigated. The afore-mentioned authors (6,7,8) had employed rat liver slices, while in the present studies rat liver homogenates were used. To permit comparison of our data with those of the other authors(6,7,8), the influence of nicotinic acid on conversion of acetate-1-C^{14} to sterols was tested in our system.

Methods and materials. The experimental procedure used to measure incorporation of MVA-2-C^{14} into sterols has been reported (9). Rat liver homogenates were prepared as described previously(9). Female and male rats weighing 200-360 g were employed. MVA-2-C^{14} (New England Nuclear Corp.) was isolated from the dibenzylethylenediamine salt (DBED); 0.5 mg MVA (0.05 μc) was added per flask. Where acetate was used, each flask contained 0.8 mg sodium acetate (1 μc). Nicotinic acid (General Biochemical Co.), isonicotinic acid, isonicotinic acid hydrazide, kynurenic acid, trigonelline, 6-OH nicotinic acid, and pyridine-3-sulfonic acid (the latter 6 compounds were obtained from Nutritional Biochemical Co.) were tested at levels of 1 mg/ml to 5 mg/ml. The excess acidity of these compounds was neutralized with KOH. Each flask contained 5 ml of rat liver homogenate, 1 mg ATP, 10 mg DPN, and 162 mg sodium succinate. Total volume per flask was 10 ml. Ribonuclease (RNAse), Worthington, 5 or 10 mg per flask and $CaCl_2 \cdot 2H_2O$ (7.5 mg per flask), where indicated, were used as "negative controls". Both of these materials have been shown previously to inhibit conversion of MVA to sterols(12,13). Homogenization and additions of ATP, DPN, and RNAse were at 4°C. The flasks were prepared by adding the homoge-