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SKELETAL MUSCLE NECROSIS FOLLOWING MEMBRANE-ACTIVE DRUGS PLUS SEROTONIN

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SUMMARY

Administration of imipramine plus serotonin (5-HT) to rats has been proposed as an animal model of Duchenne muscular dystrophy. We studied the skeletal muscle necrosis produced in male rats given 5-HT after pretreatment with imipramine, other tricyclic antidepressants, or antihistamines, which like the tricyclic antidepressants, can block neuronal reuptake of 5-HT. Following one of these agents plus 5-HT, 20 mg/kg subcutaneously (s.c.), necrosis was more severe in the soleus muscle than the quadriceps. There was no significant difference in the incidence of necrosis in the soleus and quadriceps muscles following one of these agents plus 5-HT, 100 mg/kg, intraperitoneally (i.p.). After one of these agents plus 5-HT i.p., but not 5-HT s.c., extensive necrosis was significantly more frequent and severe in the quadriceps muscle than after 5-HT s.c. Chlorpheniramine (CP) plus 5-HT, 2.5 mg/kg intravenously, produced less muscle necrosis than CP plus 5-HT s.c. or i.p. The necrosis produced by CP plus 5-HT s.c. was comparable ipsilateral and contralateral to the injection site. The necrosis following CP plus 5-HT i.p. was maximal at 24 hr and remained fairly constant until 5 days. Regeneration was prominent by 7 days. The muscle necrosis produced by CP plus 5-HT is blocked by some 5-HT blockers, e.g., methiotepin and methysergide. It is also partially blocked by denervation. The capacity of tricyclic antidepressants and antihistamines to block neuronal 5-HT reuptake tended to be negatively correlated with the capacity to potentiate the muscle necrosis they produced with 5-HT, which suggests that blockade of 5-HT uptake is not the mechanism of the pathology produced by the combined treatment. The tricyclic antidepressants and the antihistamines are "membrane stabilizers-labilizers". Other drugs which are

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"membrane stabilizers-labilizers" such as trihexyphenidyl and procaine also promoted skeletal muscle necrosis when given prior to 5-HT. It is proposed that the effects of imipramine plus 5-HT on skeletal muscle are not due to the blockade of neuronal uptake of 5-HT and subsequent vascular-induced ischemia, but reflect direct toxic effects of these agents on skeletal muscle.

INTRODUCTION

There is some evidence which suggests that serotonin (5-HT) may be involved in the etiology of Duchenne-type muscular dystrophy (DMD). In the rat, aortic ligation and small doses of 5-HT produce scattered necrosis and increased serum creatine phosphokinase (CPK) activity, both of which are characteristic of DMD (Mendell, Engel and Derrer 1971, 1972). The skeletal muscle damage has been attributed to functional ischemia. It has been shown that the initial rate of uptake of 5-HT into blood platelets from patients with DMD was less than that in normal controls and patients with other types of neuromuscular disorder (Murphy, Mendell and Engel 1973). Because platelet uptake of 5-HT could parallel neuronal reuptake, which is a major route of inactivation of 5-HT (Iversen 1974), and because excessive levels of 5-HT might lead to muscle damage by promoting functional ischemia or ischemia secondary to thrombus formation, it was cautiously proposed that this deficiency in 5-HT uptake, if generalized, could be involved in the etiology of DMD (Murphy et al. 1973). Further studies demonstrated that 3 days of treatment with imipramine, which blocks the neuronal uptake of amines in vivo and in vitro (Carlsson, Corrodi, Fuxe and Hökfelt 1969a), together with 5-HT, 100 mg/kg intraperitoneally (i.p.) 24 hr after the final dose of imipramine, produces focal areas of necrosis of skeletal muscle, especially in the quadriceps muscle and occasionally in the soleus (Parker and Mendell 1974). Imipramine alone did not produce muscle necrosis. The pattern of necrosis after imipramine plus 5-HT was said to resemble strongly the lesions of DMD. It was proposed that imipramine plus 5-HT treatment in rats produces the biochemical lesion found in patients with DMD and thus provides an excellent animal model for the study of DMD (Parker and Mendell 1974).

Because of the multiplicity of effects of most pharmacologic agents, we believed that it was necessary to conduct further studies before concluding that imipramine was acting via its effects as a blocker of 5-HT neuronal reuptake and that 5-HT was producing necrosis via an ischemic mechanism. We also investigated the effect of the route of 5-HT administration, time course, the effect of pretreatment with 5-HT blocking agents and reserpine, and the effect of prior denervation to explore the mechanism and significance of this myopathy.

METHODS

Male Sprague-Dawley rats from Sprague-Dawley, Inc., Madison, Wisconsin, weighing 150–200 g were studied. They were housed in groups of 6 and given Purina

lab chow and water ad libitum prior to and throughout the period of drug treatment.

Imipramine and other agents given to potentiate the effects of 5-HT were always administered daily for 3 days. This 3-day course of pretreatment will be referred to as the standard pretreatment protocol. The route of administration of 5-HT following the standard pretreatment protocol will be specified for each experiment. The dose of 5-HT i.p. was always 100 mg/kg, the dose of 5-HT subcutaneously (s.c.) was always 20 mg/kg. 5-HT was given 15 min after the last dose of the standard pretreatment protocol. S.c. injections were given over the right quadriceps. Muscle specimens were obtained from the right quadriceps and soleus. The quadriceps specimens from the injected leg were obtained from beneath the injection site. Intravenous (i.v.) injections were given rapidly into the exposed femoral vein under ether anesthesia. Hemostasis was achieved by pressure and Gelfoam®. In studies with amine receptor-blocking drugs, the blocker was given 15 min after the last dose of the agent given according to the standard pretreatment protocol, and 5-HT was administered 15 min after the blocker, unless otherwise noted. Also, unless otherwise indicated, rats were sacrificed 72 hr after 5-HT since at this time, pathology was as extensive as at 96 hr, the interval employed by Parker and Mendell (1974). Other details of timing and amount of drug treatment prior to sacrifice are provided in the RESULTS section. In denervation studies, the right sciatic nerves of 12 rats were cut under ether anesthesia and a 5 mm section was removed 24 hr prior to initiating drug treatment; a sham operation was performed on the leg at the same time. The incidence and extent of skeletal muscle necrosis in the sham-operated and denervated legs were compared.

At the time of sacrifice, the rats were anesthetized with pentobarbital and the soleus and a portion of the quadriceps were excised and frozen in isopentane cooled in liquid nitrogen. Frozen sections were stained with the modified trichrome reaction (Engel and Cunningham 1963) and the hematoxylin and eosin stain and examined by 2 observers. No distinction was made between fibers undergoing early necrosis or end-stage necrosis with phagocytosis. For purposes of data presentation, biopsies were assigned to one of three categories: (1) no necrosis; (2) mild necrosis ($< 1\%$ fibers were necrotic, usually scattered throughout the specimen) and (3) moderate-severe necrosis ($> 1\%$ of the fibers were necrotic, usually grouped). All sections were reviewed by 2 observers independently. The significance of the differences in incidences of the 3 categories cited above, between any 2 groups of rats was determined by a 2×3 Chi-square test. This compares both the incidence of any type of necrosis and the relative amounts of mild and moderate–severe necrosis. Inspection of the data reveals whether significant differences are likely to be due to differences in the amount of mild necrosis or moderate–severe necrosis, or both.

The following drugs and their sources were used in these experiments: imipramine and reserpine (Ciba Pharmaceutical Co., Summit, N.J.); chlorimipramine, chlorpheniramine, and pheniramine (Schering Corporation, Union, N.J.); desimipramine (Geigy Pharmaceuticals, Ardsley, N.Y.); pargyline (Abbott Laboratories, North Chicago, Ill.); methysergide (Sandoz Pharmaceuticals, Hanover, N.J.); methiotepin (Hoffman-LaRoche, Inc., Nutley, N.J.); cyproheptadine (Merck, Sharp and Dohme, West Point, Pa.); propranolol (Ayerst Laboratories, New York,

N.Y.); trihexyphenidyl (Lederle Labs, Pearl River, N.Y.); dibenzyline and chlorpromazine (Smith, Kline and French Laboratories, Philadelphia, Pa.); morphine sulphate (Merck Chemical Division, St. Louis, Mo.); serotonin creatine phosphate (Sigma Chemical Corporation, St. Louis, Mo.); procaine HCl (K & K Labs, Inc., Plainview, N.Y.).

RESULTS

Muscle pathology in untreated rats and following individual drugs

Necrotic fibers were not found in the quadriceps or soleus specimens from 30 and 18 untreated rats, respectively. We also did not observe any necrotic fibers in the soleus or quadriceps specimens from groups of 3 rats, 72 hr after receiving 3 daily doses of pargyline (75 mg/kg, i.p.), desimipramine, imipramine, chlorimipramine, or chlorpheniramine (all 25 mg/kg, i.p.) followed by saline 15 min after the last dose of pargyline or one of the amine uptake blockers. Similarly, there were no necrotic fibers in quadriceps or soleus muscle specimens from groups of 12 rats given saline for 3 days followed by 5-HT s.c. or i.p. and sacrificed 72 hr later.

Effect of route on administration of 5-HT on muscle necrosis in quadriceps and soleus

Following pretreatment with chlorimipramine, imipramine, iprindole, desimipramine (all tricyclic antidepressants), chlorpheniramine or pheniramine [antihistamines which are reuptake inhibitors (Lidbrink, Jonsson and Fuxe 1971; Korduba, Veals and Symchowikz 1973)] for 3 days in doses of 25 mg/kg i.p., approximately equal numbers of rats pretreated with each drug were given either 5-HT s.c. or 5-HT i.p. and sacrificed 72 hr later. We combined the results from all tricyclic antidepressants and antihistaminic agents for a comparison of the susceptibility of the quadriceps and the soleus and of the effects of administering 5-HT s.c. or i.p. (Table 1).

TABLE 1

COMPARISON OF NECROSIS IN QUADRICEPS AND SOLEUS FOLLOWING AMINE REUPTAKE BLOCKERS PLUS 5-HT s.c. OR i.p.

Route of 5-HT administration	Muscle	Mild necrosis		Moderate–severe necrosis		Chi-square[a]	P
		incidence	%	incidence	%		
S.C.	quadriceps	16/55	29	8/55	15	13.203	< 0.001
S.C.	soleus	24/53	45	17/53	32		
I.P.	quadriceps	11/44	25	17/44	39	12.923	< 0.001
I.P.	soleus	27/44	61	6/44	14		
S.C.	quadriceps	16/55	29	8/55	15	7.827	< 0.05
I.P.	quadriceps	11/44	25	17/44	39		
S.C.	soleus	24/53	45	17/53	32	4.680	< 0.110
I.P.	soleus	27/34	61	6/44	14		

Rats were treated with one of 6 tricyclic antidepressants (see text).
[a] Two degrees of freedom.

Necrosis was more frequent and severe in the soleus than in the quadriceps following 5-HT s.c. ($P < 0.001$). Necrosis was equally frequent in the soleus and quadriceps following the uptake blockers plus 5-HT i.p. but was more severe in the quadriceps ($P < 0.001$). Necrosis was significantly more common and severe in the quadriceps following 5-HT i.p., compared to 5-HT s.c. ($P < 0.05$). There was no significant difference in the frequency or severity of necrosis produced by 5-HT s.c. or i.p. in the soleus ($P > 0.05$).

Considering the effects of all of the tricyclic antidepressants or antihistamines administered according to the standard pretreatment protocol, plus 5-HT i.p. or s.c., the mean number of scattered necrotic fibers in specimens from the vastus or soleus

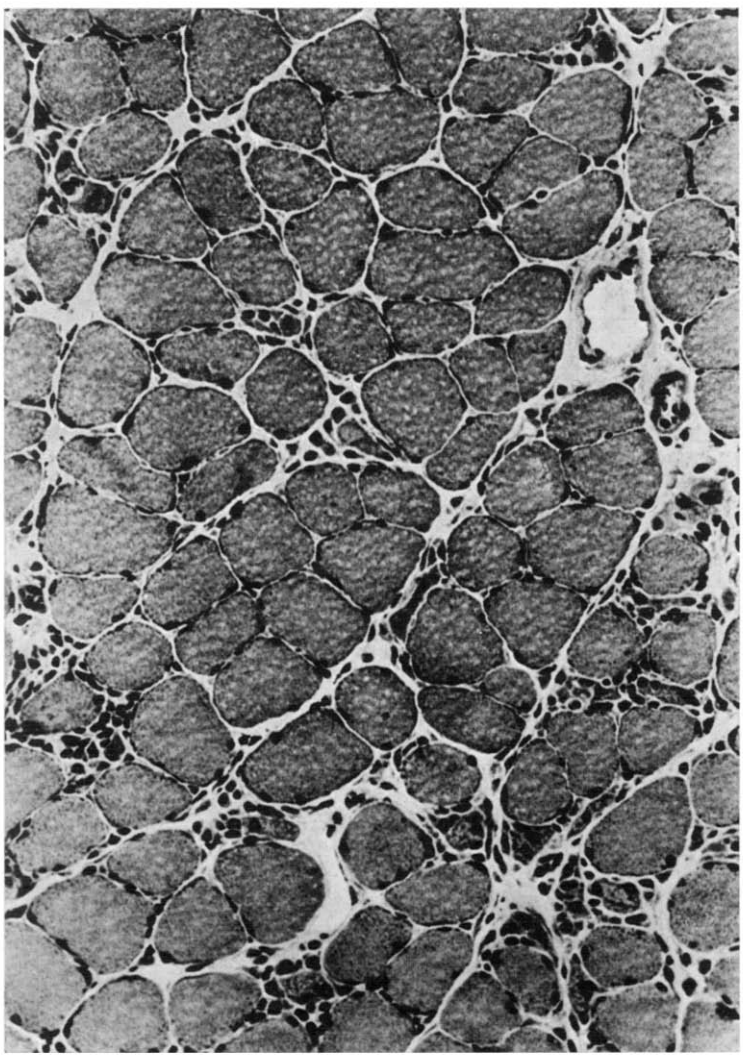

Fig. 1. Scattered necrotic fibers following chlorpheniramine plus 5-HT i.p. Quadriceps, trichrome stain, × 270.

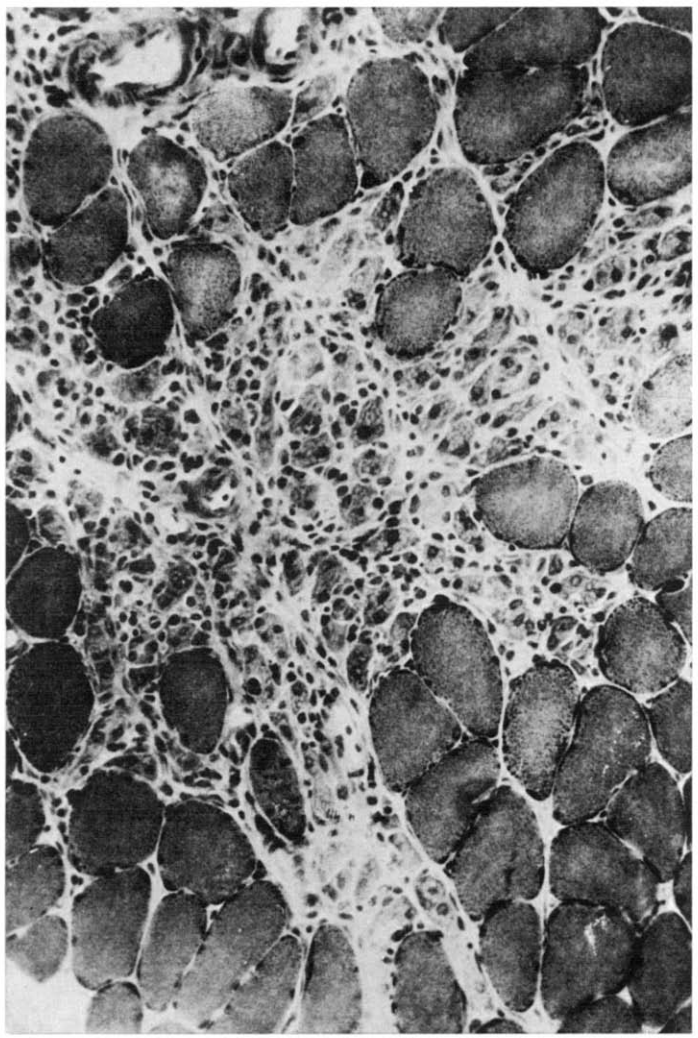

Fig. 2. Extensive necrosis of skeletal muscle following imipramine plus 5-HT i.p. Quadriceps, trichrome stain, × 270.

which had mild (scattered) necrosis was 21.4 ± SE 4.1, while the mean percentage of total fibers which were necrotic in those specimens which had moderate–severe (grouped) necrosis was 22.5 ± SE 6.1 %. The typical appearance of scattered necrosis is demonstrated in Fig. 1, while varying degrees of grouped necrosis are demonstrated in Figs. 2 and 3. There was no significant correlation between the amount of pathology found in the quadriceps and soleus of a given rat.

Following chlorpheniramine 25 mg/kg i.p. according to the standard pretreatment protocol plus 5-HT s.c. or i.p., the incidence of moderate–severe necrosis in the quadriceps and soleus muscles combined was significantly greater than that for the same pretreatment plus 5-HT, 2.5 mg/kg i.v. (Table 2).

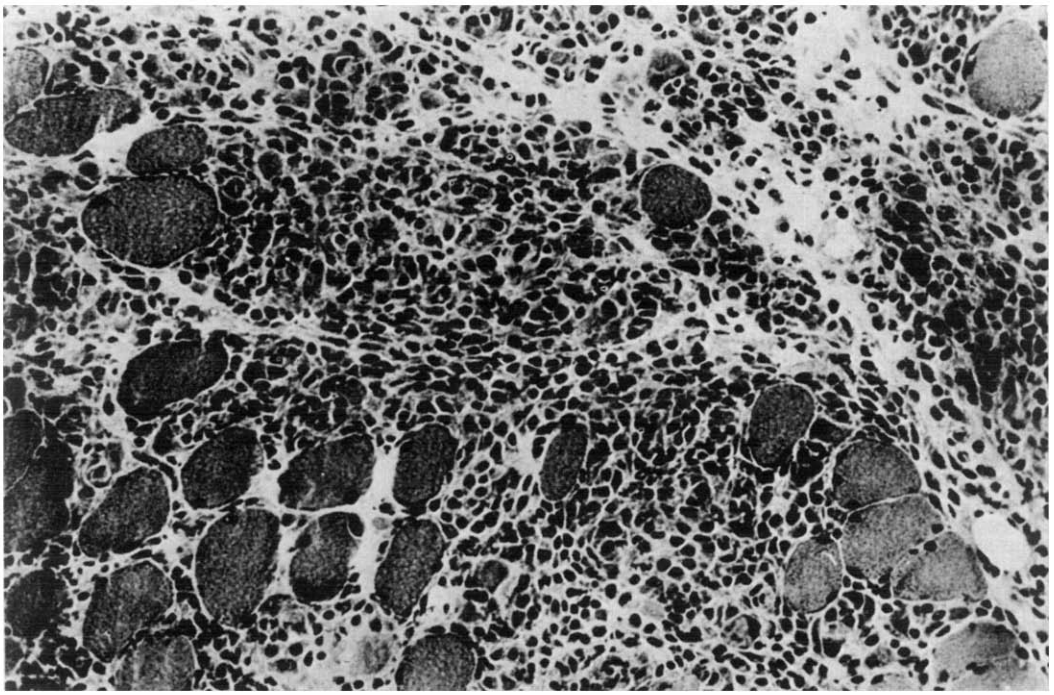

Fig. 3. Massive extensive necrosis and phagocytosis of skeletal muscle following chlorpheniramine plus 5-HT i.p. Quadriceps, trichrome stain, × 216.

TABLE 2

EFFECT OF CHLORPHENIRAMINE PLUS 5-HT i.v., s.c. OR i.p. ON SKELETAL MUSCLE NECROSIS

Treatment[a]	Dose (mg/kg)	Mild necrosis		Moderate–severe necrosis		P[b]
		incidence	%	incidence	%	
5-HT i.v.	2.5	2/10	20	0/10	0	—
5-HT s.c.	20	12/44	27	17/44	39	< 0.025
5-HT i.p.	100	10/27	39	7/27	20	< 0.05

[a] All rats were pretreated with chlorpheniramine, 25 mg/kg, i.p. for 3 days followed 15 min later by 5-HT as indicated. Rats were sacrificed 72 hr later.

[b] See METHODS for explanation of statistical analysis.

Local vs. systemic effects of 5-HT s.c.

The incidence of mild necrosis (12/44) and moderate–severe necrosis (17/44) in quadriceps and soleus specimens ipsilateral to a s.c. injection of 5-HT following the standard pretreatment protocol with chlorpheniramine was not significantly different from that contralaterally (2/12 and 4/12, respectively: Chi-square = 1.131, $P > 0.5$).

Comparison with Parker and Mendell protocol

The incidences of mild necrosis and moderate–severe necrosis in the soleus and quadriceps muscles 72 hr following our standard pretreatment protocol with imipramine plus 5-HT i.p. were not significantly different from those which are produced by the conditions employed by Parker and Mendell (1974): imipramine 10 mg/kg i.p. for 3 days followed 24 hr later by serotonin 100 mg/kg i.p. with sacrifice 96 hr later (data not presented). We confirmed the finding of Parker and Mendell (1974), that under their conditions, necrosis was more severe in the quadriceps than the soleus. Quadriceps specimens had up to 10% necrotic fibers whereas those soleus specimens with pathology had only 1–8 scattered necrotic fibers.

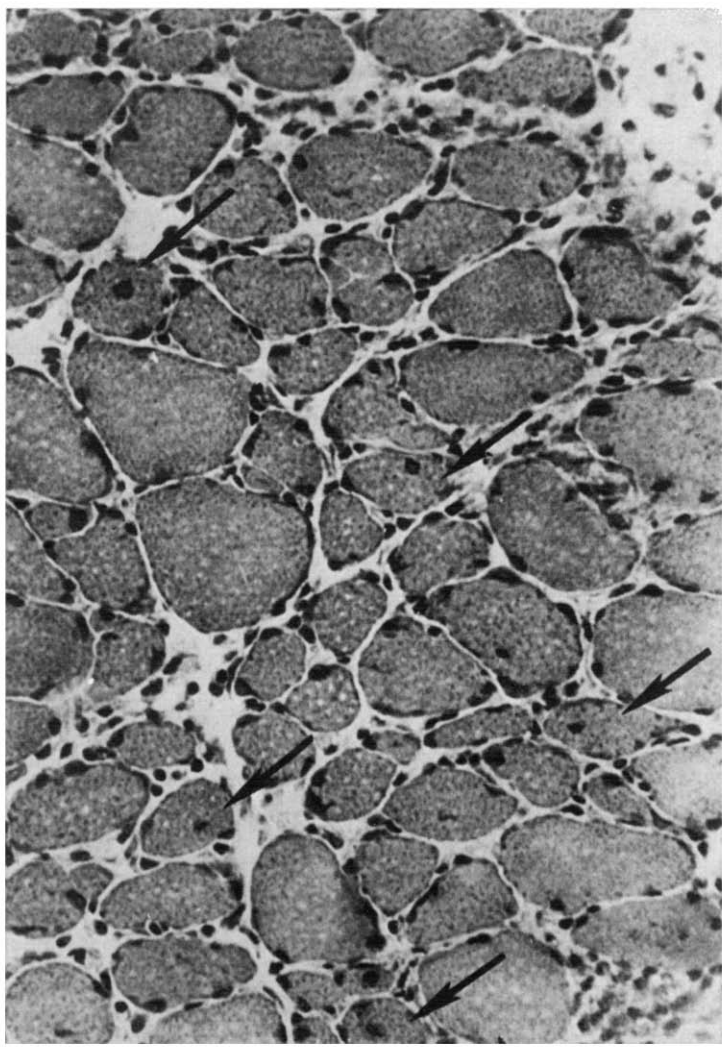

Fig. 4. Regeneration of skeletal muscle 7 days after chlorpheniramine plus 5-HT i.p. Arrows indicate fibers with central nuclei. There is a marked variation in fiber size present. Quadriceps, trichrome stain, × 324.

Time course of development of necrosis

Rats were sacrificed at intervals of 2, 24, 48, 72, 120 and 196 hr following pretreatment with chlorpheniramine according to our standard protocol plus i.p. 5-HT. The peak incidences of mild necrosis and moderate–severe necrosis were at 24 hr and remained constant until 120 hr at which time there was considerable evidence of regeneration as indicated by the presence of fibers which were basophilic and had central nuclei (Fig. 4). Necrosis was less evident and regeneration more prominent at 196 hr.

Comparison of necrosis produced by tricyclic antidepressant and other amine uptake blockers

To determine if the ability of imipramine to promote necrosis produced by 5-HT was related to its capacity to block neuronal reuptake of 5-HT, we compared the incidence and type of necrosis produced by pretreating rats with tricyclic antidepressants or antihistamines with varying capacities to block neuronal 5-HT reuptake. The skeletal muscle necrosis produced by the 5-HT reuptake blockers is given in Table 3 with the most potent reuptake blockers cited first. Iprindole is a tricyclic antidepressant which is an extremely weak blocker of neuronal or platelet 5-HT reuptake (Fann, Davis, Janowsky, Kaufmann, Cavanaugh and Oates 1974). There was a significant negative correlation (Spearman $r = -0.83$) between the rank orders for capacity to block 5-HT reuptake and the incidence of mild necrosis produced by these drugs and 5-HT. The correlation between potency of 5-HT reuptake blockade and incidence of moderate–severe necrosis was not significant ($r = 0.23$). The incidence of moderate–severe necrosis was twice as high for the two antihistamines compared to the 4 tricyclic antidepressants which all produced about the same incidence.

TABLE 3

INCIDENCE OF NECROSIS WITH VARIOUS TRICYCLIC ANTIDEPRESSANTS OR ANTIHISTAMINES PLUS 5-HT s.c. OR i.p.

Treatment[a]	Mild necrosis			Moderate–severe necrosis		
	incidence	%	rank order[b]	incidence	%	rank order[b]
Chlorimipramine	6/24	25	6	4/24	17	4.5
Chlorpheniramine	22/71	31	5	26/71	37	1
Imipramine	11/24	46	3	4/24	17	4.5
Desimipramine	16/35	46	3	5/35	14	4.5
Pheniramine	8/18	44	3	6/18	33	2
Iprindole	14/24	58	1	4/24	17	4.5

[a] Rats were given each drug at doses of 25 mg/kg i.p. for 3 days followed 15 min after last dose by 5-HT, 100 mg/kg i.p. or 20 mg/kg s.c. and sacrificed 72 hr later. Data from soleus and quadriceps are combined.

[b] Rank ordered according to greatest potency as reuptake blockers.

TABLE 4

NECROSIS FOLLOWING MEMBRANE ACTIVE DRUGS PLUS 5-HT

Pretreatment[a]	Dose (mg/kg)	Mild necrosis		Moderate–severe necrosis	
		incidence	%	incidence	%
Trihexyphenidyl	10	8/16	50	7/16	44
Chlorpromazine	5	4/6	67	0/6	0
Procaine	10	9/22	41	3/22	14
Propranolol	10	4/6	67	0/6	0

[a] Rats were given each drug for 3 days followed by 5-HT, 20 mg/kg s.c. 15 min after the last dose and sacrificed 72 hr later.

Necrosis produced by membrane active drugs plus 5-HT

It seemed possible that the membrane lytic properties of the tricyclic antidepressants and antihistamines (Langslet, Johansen, Ryg, Skomedal and Oye 1971; Seeman 1972) might be related to the necrosis produced by 5-HT. Therefore, we studied the capacity of other agents which are membrane "stabilizers" at low concentration and membrane "labilizers" at high concentration to produce muscle necrosis with 5-HT. It should be noted that the doses chosen for these studies are less than those employed with the 5-HT uptake blockers. The results are given in Table 4. Despite the lower dose of these agents, mild necrosis was produced by 5-HT plus all 4 agents studied: trihexyphenidyl, chlorpromazine, procaine and propranolol. Trihexyphenidyl plus 5-HT also produced moderate–severe necrosis as frequently as chlorpheniramine plus 5-HT, the most potent of the tricyclic drugs in this regard. With trihexyphenidyl plus 5-HT, 20–50% of the fibers in those biopsies with extensive necrosis were necrotic. For this group of agents, necrosis was present in both the soleus and quadriceps to equivalent extents.

Effect of 5-HT blockers and other amine blocking drugs on necrosis following chlorpheniramine plus 5-HT

The incidence and extent of necrosis produced by chlorpheniramine, 25 mg/kg, according to the standard protocol plus 5-HT s.c. was significantly blocked by the 5-HT blockers methiotepin and methysergide, but not by the 5-HT blockers cyproheptadine, morphine and dibenzyline (data not presented). Pretreatment with reserpine, 5 mg/kg i.p., 24 hr before or 15 min before 5-HT also did not prevent necrosis. The rationale for reserpine pretreatment is given in the DISCUSSION.

Effect of denervation on necrosis following chlorpheniramine plus 5-HT s.c.

Denervation only slightly reduced the incidence of necrosis produced by chlorpheniramine plus 5-HT s.c. (data not presented). However, the size of the areas of extensive necrosis was significantly smaller in the denervated leg (median 2–3%) than in the intact leg (median 15%) (Mann–Whitney U test, $P < 0.05$).

Necrosis produced by pargyline and pargyline plus 5-HT

Yu, Wright, Dettbarn and Olson (1974) reported that pargyline, a monoamine oxidase (MAO) inhibitor, was capable of producing skeletal muscle necrosis in rats after as little as 3 days of treatment with doses of 75 mg/kg i.p. We found 3 and 12 scattered necrotic fibers in 2 soleus specimens and none in 4 other soleus and 6 quadriceps specimens from 6 rats treated with pargyline, 75 mg/kg i.p. for 5 days. However, moderate–severe necrosis was present in the soleus of 3 of 6 rats given pargyline, 75 mg/kg i.p. for 3 days followed by 5-HT 20 mg/kg s.c. and sacrificed 72 hr later. The quadriceps muscles from 5 of the 6 rats had a few scattered necrotic fibers.

DISCUSSION

The studies reported here confirm the report of Parker and Mendell (1974) that imipramine potentiates the toxic effect of 5-HT on skeletal muscle. However, two of our findings cast doubt on their hypothesis that the greater necrosis in the quadriceps than the soleus is due to intrinsic properties of the two muscles. First, when 5-HT was injected s.c. over the quadriceps, there was a significantly higher incidence of necrosis in the soleus than the quadriceps. Second, following the pretreatment schedule which we employed, there was no significant difference in the incidence of necrosis in the quadriceps and soleus produced by 5-HT i.p. although the necrosis that did develop in the quadriceps was more frequently of the moderate–severe type than in the soleus. This suggests that the experimental conditions determined which muscle is affected rather than intrinsic properties of either muscle.

The greater necrosis in the soleus than the quadriceps after the tricyclic antidepressants of antihistamines plus 5-HT s.c. has two other implications. It indicates that diffusion of 5-HT into the muscle underneath the injection site was less important in producing necrosis than absorption into the circulation and delivery to muscle via the blood stream. This can be compared with local anesthetics given s.c. which produce muscle necrosis only under the injection site (Benoit and Belt 1970, 1972). Second, it may also be an indication that muscle activity is relevant to the necrosis produced by these treatments since the soleus is a tonic muscle in which motor units in standing rats are continuously active whereas the quadriceps is largely a phasic muscle. The role of muscle activity in producing necrosis following imipramine-type drugs and 5-HT is further indicated by the partial inhibition of necrosis afforded by denervation.

If muscle activity is important to the necrosis following imipramine plus 5-HT, this might suggest that anoxia secondary to vascular insufficiency is the immediate cause of necrosis. This hypothesis is consistent with the observation that 5-HT i.v. following chlorpheniramine pretreatment produced much less necrosis than 5-HT s.c. or i.p. since i.v. 5-HT can increase muscle blood flow (Takacs and Vajda 1963). However, there are several arguments against this hypothesis. The limited necrosis produced by 5-HT i.v. may not be too significant since only small doses of 5-HT could be given by this route relative to the doses administered s.c. or i.p., and the

i.v. 5-HT would be subject to rapid oxidation by liver MAO and uptake by various organs (Axelrod and Inscoe 1963). Secondly, in the myopathy produced by aortic ligation, which has also been attributed to anoxia, denervation, which eliminates muscle activity completely, prevented muscle necrosis and maximal necrosis did not develop until 96 hr after aortic ligation (Mendell et al. 1971; Karpati, Carpenter, Melmed and Ersen, 1974). The myopathy due to chlorpheniramine plus 5-HT is fully developed at 24 hr and is only partially blocked by denervation. Direct studies of muscle blood flow or oxygen tension in muscles might clarify the role of anoxia in this myopathy.

The fact that pretreatment with methiotepin and methysergide, both 5-HT blockers, blocked the necrosis produced by chlorpheniramine plus 5-HT s.c. tends to support the conclusion that the necrosis produced by the tricyclic antidepressant or antihistamines plus 5-HT is, in fact, due to a specific effect on 5-HT receptors. Cyproheptadine, morphine and dibenzyline, although potent blockers of some effects of 5-HT (Gaddum and Hameed 1954; Gyermek 1961) may be too weak as blockers of this effect of 5-HT upon whose nature we will speculate subsequently. A specific serotonergic effect is also supported by the finding that pargyline, an MAO inhibitor, also potentiates the muscle necrosis following 5-HT. Pargyline inhibition of MAO may promote necrosis in skeletal muscle by preventing 5-HT catabolism in liver and other organs, as well by inhibiting the small amount of oxidative deamination of 5-HT of which skeletal muscle itself is capable (Arora and Meltzer, unpublished data). Our inability to confirm the findings of Yu et al. (1974) that pargyline itself produced necrosis of a soleus muscle is difficult to explain.

We studied the effect of reserpine pretreatment with two objectives: (1) to determine if 5-HT uptake and storage into tissues sensitive to reserpine was important to the necrosis produced by the imipramine-type drugs plus 5-HT; (2) to determine if hypotension, which should be potentiated by reserpine, was an important contributor to the necrosis produced by imipramine-type drugs plus 5-HT. Reserpine given 15 min before 5-HT and 24 hr before 5-HT reduces the overall uptake and storage of 5-HT by neuronal amine storage granules, blood platelets and other tissues (Shore 1966) and should leave more of the exogenous 5-HT free to react with 5-HT receptors. This type of mechanism is believed to account for the suprasensitivity to catecholamines observed after reserpine treatment (Shore 1966). The 5-HT released by reserpine from storage sites is largely metabolized by MAO before it leaves neurons and other storage sites and should not influence the extent of necrosis produced by chlorpheniramine plus 5-HT. The fact that reserpine neither blocked nor promoted the necrosis due to chlorpheniramine plus 5-HT is an indication that uptake of 5-HT into storage granules for later release is not required for the development of necrosis. Since making more exogenous 5-HT available by preventing its uptake and storage does not enhance necrosis, this suggests that the available serotonin is not the limiting factor to the amount of necrosis which takes place. Finally, since the reserpine-treated rats should develop hypotension which should further diminish blood flow to muscle, the fact that no greater necrosis occurred after reserpine is further evidence that decreased blood flow is not the major factor in causing skeletal muscle necrosis.

Our studies of the effects of a series of 5-HT reuptake blockers of varying potency on muscle necrosis cast doubt on the hypothesis of Parker and Mendell that the relevant effect of imipramine is its capacity to block 5-HT uptake by neurons. The doses of tricyclic antidepressants and antihistamines we employed are those usually used in rats in studies designed to achieve blockade of 5-HT uptake in vivo (Carlsson et al. 1969a; Carlsson, Jonason, Lindquist and Fuxe 1969b; Carlsson and Lindquist 1969; Lidbrink et al. 1971). Although there are differences in molecular weight of the tricyclic antidepressant and antihistaminic reuptake blockers, these are small enough that it is unlikely that differences in molar concentrations accounted for the differences in their effectiveness in our studies. Chlorimipramine is a much better inhibitor of the neuronal reuptake of 5-HT than is imipramine (Carlsson et al. 1969a,b; Lidbrink et al. 1971; Ross and Renyi 1975). On the other hand, desimipramine is a weaker inhibitor of 5-HT reuptake than is imipramine (Carlsson et al. 1969a; Lidbrink et al. 1971). Pheniramine is a much weaker uptake blocker than chlorpheniramine (Korduba et al. 1973). If the necrosis produced by imipramine plus 5-HT is due to inhibition of 5-HT reuptake by neurons, then the necrosis produced by chlorimipramine or chlorpheniramine plus 5-HT should be greater than that produced by imipramine or pheniramine plus 5-HT. Desimipramine plus 5-HT should produce less necrosis than imipramine plus 5-HT. These predictions were not all confirmed by our studies. Desimipramine tended to be more effective rather than less effective than imipramine or even chlorimipramine in promoting necrosis following 5-HT. Chlorimipramine, which should have been among the most effective of the tricyclic drugs in producing muscle necrosis, was among the least effective. Pheniramine which also should have been relatively inactive was as effective as chlorpheniramine in potentiating the effects of 5-HT. Iprindole, which is a tricyclic antidepressant like imipramine, but has no capacity to block 5-HT uptake (Fann et al. 1974) was, in fact, the most potent agent in producing mild necrosis when given as pretreatment prior to 5-HT. These results suggest that blockade of *neuronal* uptake of 5-HT by the tricyclic antidepressants and antihistamines is not the relevant factor in the interaction with 5-HT to produce skeletal muscle necrosis. However, the effect of these agents on 5-HT uptake by tissues other than nerve, perhaps skeletal muscle itself, could be the important factor in the development of necrosis and the relative potency of these agents on 5-HT uptake by various organs has not been studied.

If the imipramine-type agents are not acting by blocking neuronal reuptake of 5-HT and if the 5-HT effect is not primarily vascular, what then is the mechanism of the necrosis produced by these agents? Fenichel, Dettbarn and Newman (1974) have presented evidence which suggest acetylcholine excess can produce muscle necrosis. There is evidence that 5-HT can release acetylcholine at the motor nerve ending (Dretchen, Ghoneim and Long 1972) and that this release is blocked by methysergide, as was the case with the necrosis in these studies. The tricyclic antidepressants, however, appear to *decrease* the amount of acetylcholine liberated from the motor nerve terminals (Lermer, Avni and Bruderman 1971; Chang and Chuang 1972). Furthermore, the acetylcholine myopathy is completely blocked by prior denervation (Fenichel et al. 1974). Thus, it appears unlikely that this effect of 5-HT is important for the muscle necrosis.

The myopathy produced by the tricyclic antidepressant, antihistamines, trihexyphenidyl and procaine plus 5-HT, could be due to the membrane-destabilizing action of these drugs. Abood, Kimizuka, Rogeness and Biel (1963) and Langslet et al. (1971) have presented evidence that the tricyclic drugs are "membrane stabilizers" at low concentration, which generally means that they are membrane labilizers at high concentrations (Seeman 1972). It is not known if concentrations of these drugs sufficiently high as to produce membrane labilization were achieved in these experiments. We have found that imipramine at theses doses can by itself significantly elevate rat serum CPK activity and that this increase can be further augmented by serotonin (Meltzer, H. Y., unpublished data). The membrane stabilizing-labilizing effects of imipramine on rat heart are also shared by the local anesthetic, lidocaine (Langslet et al. 1971). As previously mentioned, local anesthetics can produce skeletal muscle necrosis (Benoit and Belt 1970, 1972). An effect of imipramine to destabilize the muscle membrane as the cause of the necrosis is supported by the studies of Canal, Frattola, Scarlato and Pavani (1973) who found that Triton X-100, a membrane-lytic agent, could substitute for 5-HT in producing skeletal muscle necrosis after aortic ligation. We have found that imipramine at concentrations between 1 m*M* and 0.1 m*M* can promote efflux of CPK from the rat extensor digitorum longus in vitro and that this efflux is potentiated by 5-HT (Meltzer, H. Y., unpublished observations).

The mechanism by which 5-HT promoted necrosis after pretreatment with membrane stabilizer-labilizer drugs could be due to a direct interaction of 5-HT and these agents at the sarcolemma which interfere with the capacity of the sarcolemma to maintain normal intracellular ionic concentrations of calcium and other cations. Serotonin has been demonstrated to promote calcium uptake by smooth muscle and other tissues (Wooley 1958). The cardiac necrosis produced by isoproterenol (Bloom and Davis 1972) as well as the necrosis due to local anesthetics (Benoit and Belt 1970, 1972) has been attributed to inhibition of calcium uptake by the sarcoplasmic reticulum. The effect of the increased intracellular calcium might be to activate or inhibit one or more adenosine triphosphatases which in turn might lead to decreased availability of adenosine triphosphatase (ATP). Inadequate amounts of ATP could produce many deleterious effects on the muscle cell. Studies of intracellular 5-HT, calcium, sodium, potassium, and high energy phosphate compounds in skeletal muscle after imipramine plus 5-HT are indicated.

Other possible causes of the muscle necrosis after pretreatment with the membrane stabilizer-labilizer drugs are that they: (1) might enable 5-HT to penetrate the cell in larger amounts than usual via enhanced passive diffusion; (2) diminish efflux of any 5-HT which might enter the cell; or (3) augment active uptake of 5-HT. 5-HT uptake by muscle after i.v. 5-HT is usually slight (Axelrod and Inscoe 1963), but might be greater if neuronal reuptake is blocked or because of the effect of the membrane stabilizer-labilizer drugs in vitro. 5-HT is a relatively poor substrate of rat skeletal muscle monoamine oxidase (Arora and Meltzer, unpublished data) which would mean that after uptake by skeletal muscle, oxidative catabolism of 5-HT would proceed slowly. However, we have studied the uptake of 5-HT into

muscle in vitro and found that it is not augmented by the tricyclic drugs (Stahl and Meltzer, unpublished data).

The results reported here are not consistent with the theory that administration of imipramine plus 5-HT to rats is a good model for human DMD if it is assumed that such a model should include a defect in the reuptake of 5-HT, anoxia secondary to diminished blood supply, and more pathology in proximal rather than distal muscles. Nevertheless, this is an interesting model for further study in that it appears to afford an opportunity to study the role of the sarcolemma in muscle injury and provides a rationale for further study of the toxic effects of 5-HT on skeletal muscle.

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