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Effect of Age, Clonidine or Propranolol on Behavioral Toxicity Induced with Digitoxin in Mice[1]

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PALFAI, T. AND V. H. FELLEMAN. *Effect of age, clonidine or propranolol on behavioral toxicity induced with digitoxin in mice.* PHARMAC. BIOCHEM. BEHAV. 17(3) 399–404, 1982.—In three experiments, behavioral toxicity induced by 5 different dose levels of digitoxin was investigated in mice that were treated with either clonidine or propranolol or were either young (70–140 days), middle aged (240–260) or old (450–600). Observed on four drug-induced behavior categories, it was found that both clonidine and propranolol attenuated the toxic effects of relatively low dose levels of digitoxin, while at higher digitoxin dose levels only propranolol was able to reduce significantly the ED_{50} and the LD_{50}. The results were discussed in terms of adrenergic mechanisms mediating digitoxin toxicity. Aged mice were found to be the least and middle aged the most resistant to the toxic effects of digitoxin.

Digitoxin Digitalis Behavioral toxicity Cardioglycosides Clonidine Propranolol

NEARLY two hundred years have passed since Withering described the effects of the foxglove, *Digitalis purpurea* [22]. Nevertheless, research and clinical application of the cardiac glycosides still continue with vigor [13, 14, 15, 16, 19]. This interest is primarily due to the ability of the digitalis glycosides to increase the force and velocity of contraction of the heart [5, 12, 15, 19, 22]. It is also this effect that makes digitalis the principle pharmacologic treatment for congestive heart failure [15,19].

In spite of the therapeutic efficacy of the digitalis compounds, following their administration toxicity develops in about 8–35% of the patients [13,19]. This observation obviously limits the clinical use of digitalis [13, 15, 17, 19]. Even when serum levels are monitored in patients, impaired renal function, drug-drug interactions, thyroid problems, the differential action of the sympathetic nervous system, the nature of the cardiac tissue, abnormal concentrations of electrolytes, psychological stress, age, genetic differences, etc. [1, 11, 13, 15, 16, 17, 18, 19, 20, 21, 23] all contribute to the drug's low therapeutic index. That is, the clinical efficacy of digitalis and its lethal toxicity are separated only by a relatively narrow margin [15, 17, 19]. It is, therefore, especially important to identify as many variables as possible that might increase or decrease the likelihood of digitalis toxicity.

The majority of publications on digitalis toxicity focus on cardio-toxicity resulting from the activation of the adrenergic nervous system, the adrenals and/or heart tissue [2, 3, 5, 6, 8, 9, 10, 13, 14, 20, 22]. From these studies, it appears that the digitalis-induced lethality may, in fact, be due to heart failure. Unfortunately, less attention has been given to the extracardial and especially the behavioral toxicity of digitalis. Namely, studies in behavioral toxicity have often yielded important information on variables that influence the toxicity of substances at dose levels below which they produce organ toxicity [7]. While behavioral studies lack the precision in explaining the mechanism by which toxicity occurs, they can provide readily attainable descriptive information about variables affecting toxicity. The purpose of the present series of experiments was, therefore, to investigate the effects of several variables as they influence the development of behavioral toxicity induced by digitoxin, a non-polar digitalis glycoside with intermediate onset of action [4,15].

EXPERIMENT 1

In the first experiment, we investigated a broad range of digitoxin dose levels as they affected gait, induced tremors or convulsions, produced a loss in the righting reflex and/or resulted in lethality. Although principally only these four behavioral categories were used to assess the onset of the drug-dosage effects, the animals were observed throughout the experiment. Other behavioral phenomena (e.g., passivity, alertness, etc.) induced by the drug treatment were also noted.

METHOD

Animals

A total of 153 males, 70–140 day old Swiss albino mice

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TABLE 1

CUMULATIVE PERCENT OF ANIMALS SHOWING AFFECTED GAIT (AG), TREMOR OR CONVULSION (TC), LOSS OF RIGHTING REFLEX (LRR) OR LETHALITY (L)

Dose (mg/kg)	n	AG	TC	LRR	L
5	29	17	3	3	3
10	25	56	28	24	24
20	36	78	59	45	39
40	38	88	68	65	60
80	25	100	92	92	88

were used. The mice were obtained from the breeding colony of Syracuse University, 101 Stevens Place, Psychology Research Laboratories. The original animal stock was obtained from CD-1 strain, Charles River Mouse farms, Wilmington, Massachusetts. The mice were housed in temperature- and humidity-controlled colony rooms, in Econo plastic cages, four to a cage and a 12-hr, day/night cycle was in effect. The mice were fed Purina Chow No. 5001, and both water and food was available ad lib throughout the experiment.

Procedure

Drug treatment. Digitoxin was obtained from the Sigma Chemical Company, St. Louis, MO. Drug Solutions were prepared fresh daily by mixing 2 drops of Tween 80 to the digitoxin powder and suspending it in distilled water. All drug concentrations were prepared so that the volume for each investigated dose level could be given as 10 ml/kg body weight. Five dose levels were used. They were 5 (n=29), 10 (n=25), 20 (n=36), 40 (n=38), and 80 mg/kg (n=25). The injections were given IP between 10:00–2:00 midday. Following the injection, the animal was placed in the sterolit covered floor of its home cage where it was observed for the next four hours. Subsequently, for the next 10 days the animals were observed every 24 hours for 5 minutes and their behaviors were recorded.

Behavior Measures. Principally, the animals were observed on four drug-induced dependent measure categories: affected gait, occurrence of tremors or convulsions, loss of righting reflex, and lethality. These categories were chosen because first, pilot data indicated behavioral consistency in the various groups on these measures and second, because they reflected an increasing degree of toxicity. That is, lethality included the other three categories: loss of righting reflex occurred after the animals had exhibited tremors or convulsions and affected gait; and animals that showed tremors or convulsions had invariably demonstrated affected gait. The dependent measure was the number of animals per group exhibiting these effects over days.

RESULTS AND DISCUSSION

The results are expressed as the cumulative percent of animals showing the onset of affected gait, tremors or convulsions, impaired righting reflex, or lethality per group and are shown in Table 1. Apart from 1 animal that died in the 5 mg/kg group (probably due to a missed injection, for not only

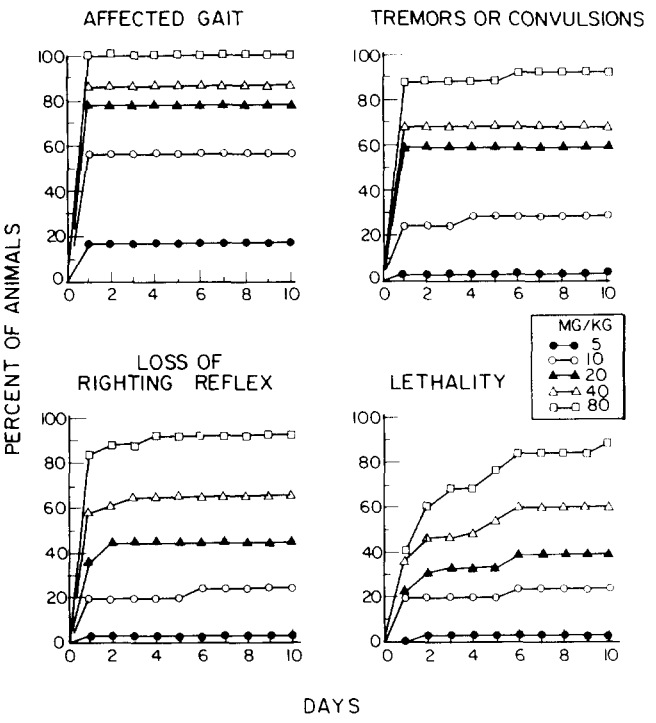

FIG. 1. Cumulative percent of animals showing affected gait, tremors or convulsions, loss of righting reflex and lethality.

did the animal die within the first hour of the injection but subjects in this group did not show affected gait), the Table shows a very orderly development of toxicity as function of the dosage. Figure 1 shows the cumulative percentages of animals per groups by days. It is interesting to note that gait was affected within the first 24 hrs following the injections, i.e., the onset of this effect was maximal within 24 hrs. The estimated ED_{50} for affected gait was 8 mg/kg. The onset of tremors or convulsions occurred within the first day among the three higher dosage groups with ED_{50} of 18 mg/kg. The onset of the loss of righting reflex was somewhat more gradual occurring on the average after the 3rd or 4th day. The ED_{50} for this category was 32 mg/kg. Finally, the cumulative percentage of lethality plateaued on Day 6 in most of the dosage groups. This appeared to be a particularly critical day since in the lower dosage groups (10, 20 and 40 mg/kg) the animals improved continuously after this day. By the 10th day, apart from the bodyweight loss, many of them were showing negligible toxic drug effects. Although probably unrelated, the phenomenon coincided with the physiologic half life of the drug in humans [3]. The LD_{50} after 6 days was 34 mg/kg.

EXPERIMENT 2

Older people comprise about 10–12% of the population of the U.S., but they receive more than 25% of all prescription drugs [1,23]. The disproportioned use of drugs by the elderly is due to an increase of physical illness, psychiatric disorders and impaired general physiologic function in old age. However, these very reasons might be responsible for the unusually large number of side effects produced by cardiac glycosides in the elderly and that often therapeutic dosages

TABLE 2

CUMULATIVE PERCENT OF ANIMALS SHOWING AFFECTED GAIT (AG), TREMOR OR CONVULSION (TC), LOSS OF RIGHTING REFLEX (LRR) OR LETHALITY (L), FOLLOWING 5 DIFFERENT DOSE LEVELS OF DIGITOXIN

		Dose				
Category	Age	5 (n)	10 (n)	20 (n)	40 (n)	80 (n)
AG	70–140	17 (29)	56 (25)	78 (36)	88 (38)	100 (25)
	230–260	0 (15)	74 (15)	80 (15)	100 (15)	100 (15)
	450–600	7 (14)	70 (15)	92 (14)	100 (13)	—
TC	70–140	3	28	59	68	92
	230–260	0	13	40	67	94
	450–600	7	47	85	100	
LRR	70–140	3	24	45	65	92
	230–260	0	20	40	54	74
	450–600	7	33	64	85	
L	70–140	3	24	39	60	88
	230–260	0	7	40	40	74
	450–600	7	33	57	85	

produce toxicity [23]. Since digitalis preparations are used by 5% of the geriatric population [1], it is very important to investigate the effect of age on digitalis toxicity in a systematic fashion. In this experiment, three age groups of mice were treated with 5 different dosages of digitoxin.

METHOD

Animals

Three age groups of mice (70–140, 230–260, 450–600 days) bred, housed and maintained in our colony as described previously were used.

Procedure

Drug treatment and behavioral testing were the same as described in Experiment 1. In actuality the two older age groups of animals were run simultaneously with the animals of Experiment 1, but for the clarity of presentation the experiment is dealt with as a separate study. All groups received 5, 10, 20, 40 or 80 mg/kg of digitoxin with the exception of the 450–600 day group. These animals were not given 80 mg/kg because already at the 40 mg/kg dose level, high mortality occurred. This allowed us to increase the number of animals in the other four dose levels in the aged animal groups.

RESULTS AND DISCUSSION

The results of this experiment are shown in Table 2. The Table shows the cumulative percentages of animals exhibiting a drug effect in each of the four measurement categories for the 3 age groups. As can be seen at the two lower dose levels, there was not much difference among the age groups in any of the four measurement categories, although a consistently higher number of animals exhibited the drug effects in the oldest group. This was especially the case with lethality when after a 10 mg dose 33% of the older animals died, while only 7% of the 230–260 day old animals died. A more obvious difference among the age groups could be observed following 20 or 40 mg/kg dosages. As shown in Fig. 2, the

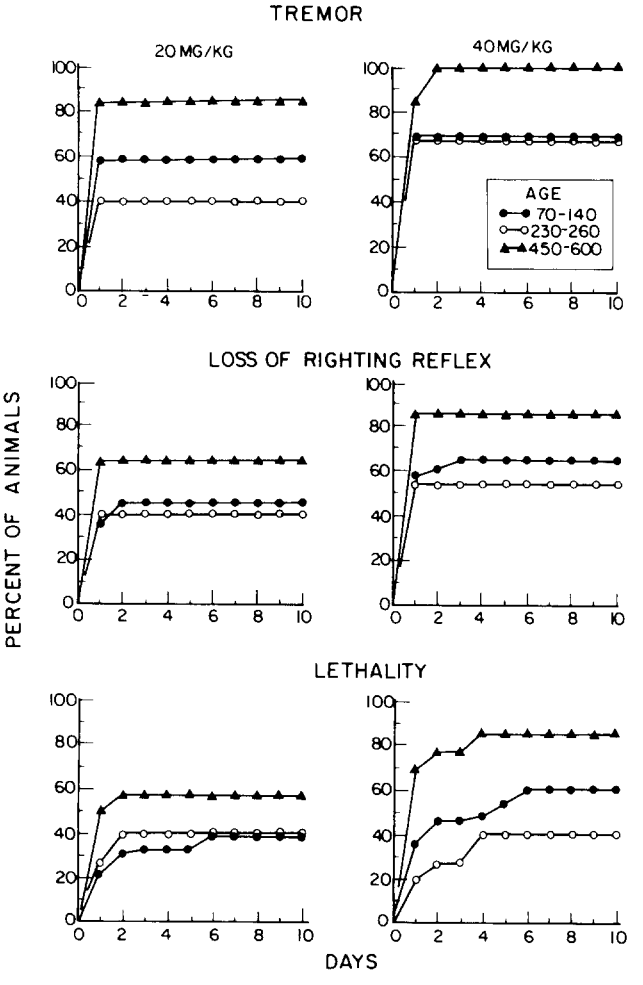

FIG. 2. Cumulative percent of animals exhibiting a drug effect in each of the four measurement categories for the 3 age groups.

TABLE 3

CUMULATIVE PERCENT OF ANIMALS SHOWING AFFECTED GAIT (AG), TREMOR OR CONVULSION (TC), LOSS OF RIGHTING REFLEX (LRR) OR LETHALITY (L), FOLLOWING 5 DIFFERENT DOSE LEVELS OF DIGITOXIN IN PROPRANOLOL (10 mg/kg) OR CLONIDINE-TREATED (0.5 mg/kg) MICE

		Digitoxin Dose				
Category	Drug	5 (n)	10 (n)	20 (n)	40 (n)	80 (n)
AG	Control	17 (29)	56 (25)	78 (36)	88 (38)	100 (25)
	Propranolol	0 (9)	17 (12)	85 (13)	92 (25)	100 (24)
	Clonidine	10 (21)	35 (20)	80 (20)	90 (20)	100 (20)
TC	Control	3	28	59	68	92
	Propranolol	0	8	54	68	84
	Clonidine	10	10	60	65	100
LRR	Control	3	24	45	65	92
	Propranolol	0	0	39	40	76
	Clonidine	10	5	55	55	100
L	Control	3	24	39	60	88
	Propranolol	0	0	31	36	50
	Clonidine	10	5	50	50	100

development of toxicity in the tremor or convulsions, loss of righting reflex and the lethality categories was readily observable in all instances of the oldest group when compared to the 230–260 day old group. The animals in this latter group appeared to be even more resistant to the toxic effects of digitoxin than in the 70–140 day old group. The development of the symptoms, as shown in Fig. 2, appeared to be the same across the age groups. As can be seen in older animals, the symptoms occurred more frequently but did not occur earlier or later than in the younger animals. Digitoxin was simply more toxic in old than in middle age or younger animals. This effect was not due to heavier body weight of older animals because the 230–260 day old animals actually weighed slightly more and thus received more net amount of digitoxin than either the younger or the older animals. The results are in agreement with other publications [13, 19, 20, 23] and certainly are in line with clinical observation that in the aged, lower dose levels of digitalis glycosides produce more toxicity than in the middle aged or the young.

EXPERIMENT 3

A considerable body of literature exists which implicates the sympathetic nervous system and especially the catecholamines in digitalis toxicity [3, 6, 8, 9, 10]. A frequently adopted research strategy to demonstrate this to be the case is to utilize drugs known to interfere with efferent adrenergic functioning at each level of neuronal transmission. For example, drugs such as clonidine might be used to depress central sympathetic outflow; ganglionic blockers to interrupt transmission of sympathetic ganglia; rauwolfias to impair presynaptic action; and, β-adrenergic blockers to affect postsynaptic terminals [8, 9, 10]. As an excellent review concludes [8], it appears that a variety of anti-adrenergic pharmacologic manipulations can lead to protection from digitalis toxicity.

The purpose of the present experiment was to compare the possible protective effects of a mainly centrally (clonidine) and a primarily peripherally (propranolol) acting antiadrenergic agents on behavior toxicity induced by digitoxin.

TABLE 4

Category	Age Condition	n	ED_{50} (mg/kg)	LD_{50} (mg/kg)
AG	70–140 Control	153	8	
	70–140 Clonidine	101	12	
	70–140 Propranolol	84	14	
	230–260 Control	60	7	
	450–600 Control	56	7	
TC	70–140 Control	153	18	
	70–140 Clonidine	101	27	
	230–260 Propranolol	84	19	
	230–260 Control	60	36	
	450–600 Control	56	16	
LRR	70–140 Control	153	32	
	70–140 Clonidine	101	27	
	70–140 Propranolol	84	56	
	230–260 Control	60	38	
	450–600 Control	56	16	
L	70–140 Control	153		34
	70–140 Clonidine	101		31
	70–140 Propranolol	84		80
	230–260 Control	60		56
	450–600 Control	56		16

METHOD

Animals

A total of 189 male, 70–140 day old mice of the same description as before were maintained as indicated previously.

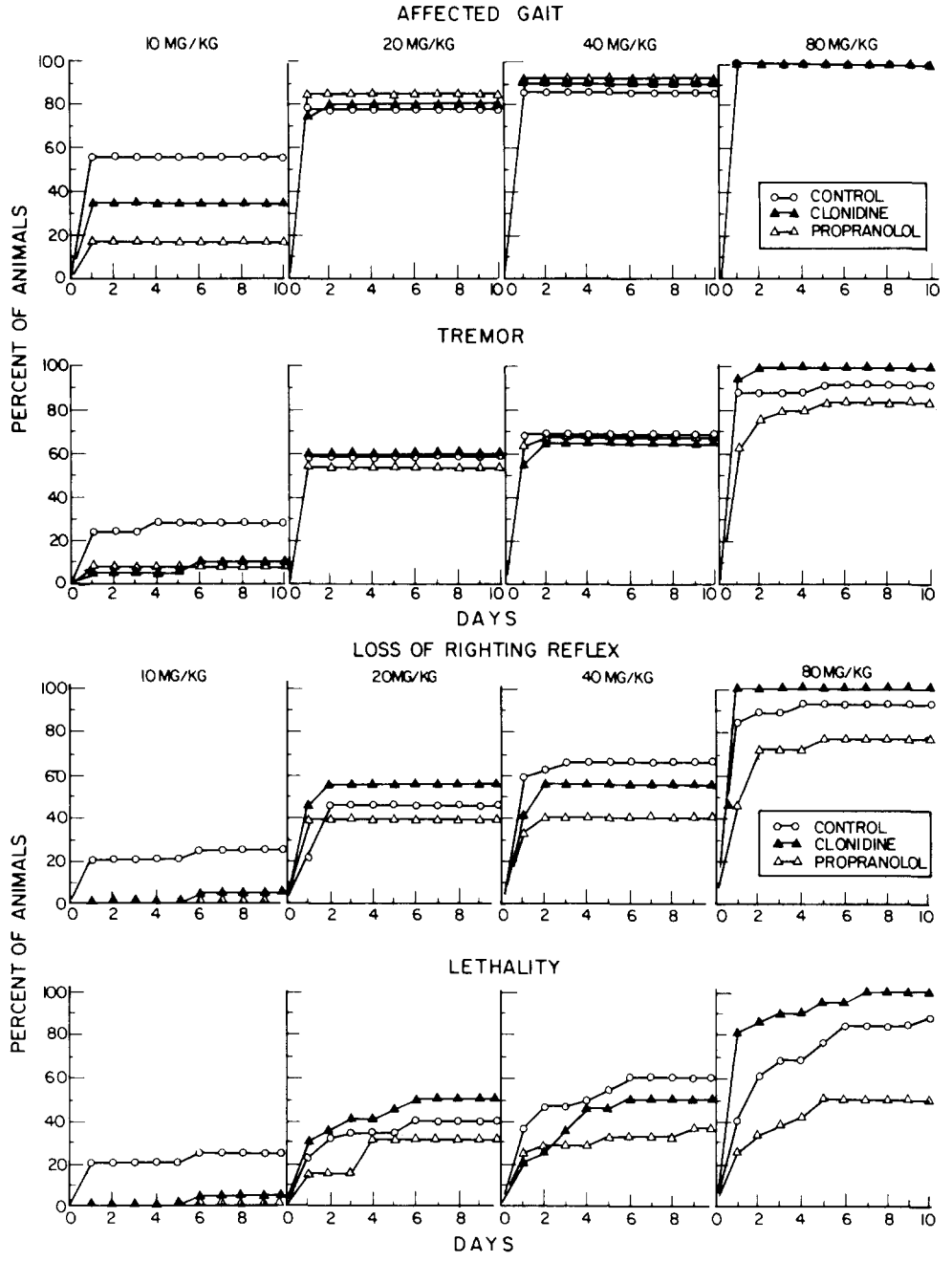

FIG. 3. Cumulative percent of animals showing symptoms after various digitoxin doses and treatment with propranolol, clonidine and the drug vehicle.

Procedure

Clonidine hydrochloride (Boehringer Ingelheim, Ltd.) was dissolved in physiologic saline and injected IP 2 and 24 hours following an IP injection of either 5, 10, 20, 40 or 80 mg/kg digitoxin. Propranolol hydrochloride (Ayerst Laboratories Inc.) was dissolved in distilled water and injected SC 3 and 5 hrs following an IP injection of either 5, 10, 20, 40 or 80 mg/kg digitoxin. The solutions were made fresh daily and the dosages (clonidine, 0.5 mg/kg; propranolol, 10 mg/kg) were selected on the basis of a maximal dose level without behaviorally observable effects. The animals were observed as described previously.

RESULTS AND DISCUSSION

The results are shown in Table 3. The Table shows the percentage of animals exhibiting behavioral symptoms and lethality following 5 different dose levels of digitoxin in propranolol- or clonidine-treated mice. A complex, but rather interesting toxicity pattern of the drug/digitoxin combination emerges. At the 5 and 10 mg/kg digitoxin dose levels, both

propranolol and clonidine attenuated the toxic effects of digitoxin in all four of the measurement categories; propranolol appeared to be more efficient than clonidine. Thus, the biological mechanisms underlying digitoxin-induced affected gait, tremors or convulsions, impaired righting reflex or lethality might have both a central and a peripheral sympathetic component. At higher digitoxin dose levels (20, 40 or 80 mg/kg) neither of these compounds attenuated the occurrence of the digitoxin-induced affected gait or tremors or convulsions which might mean that toxicity in these behavior categories does not involve only adrenergic mechanisms. A rather striking effect is, however, the considerable efficacy of propranolol but not clonidine to attenuate impaired righting reflex and especially lethality induced by higher doses (20, 40, 80 mg/kg) of digitoxin. A reasonable speculation concerning the effects of the drug combinations on these measurement categories might be that the physiologic mechanisms underlying the righting reflex but especially those of digitoxin induced lethality in mice involve peripheral postsynaptic adrenergic receptors probably at the heart tissue. For comparative purposes, Table 4 shows all ED_{50}s and LD_{50}s from the three experiments. Finally, Fig. 3 shows that if the digitoxin-induced toxic symptoms occurred, they developed approximately at the same rate following in both clonidine or propranolol treated mice as in the control animals. This observation suggests that the protective effect of these compounds was not simply due to a postponement of the toxic effect of digitoxin.

GENERAL DISCUSSION

In a series of experiments the behaviorally toxic effects of digitoxin in young, middle aged and old, or propranolol- or clonidine-treated mice were investigated. In clear agreement with clinical observations [10,23] digitoxin was found to be the most toxic in old followed by young and middle aged subjects respectively. Digitoxin's behavioral toxicity appeared to be mediated by adrenergic mechanisms involving both a central sympathetic and a peripheral postsynaptic adrenergic component following low dosages. The mechanism underlying lethality induced by digitoxin appeared to involve peripheral postsynaptic adrenergic mechanisms, probably at the heart, since it could effectively be attenuated following the administration of propranolol.

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