Hemodynamic Alterations in Isoproterenol-Induced Cardiac Arrhythmias in Corticoid-Treated Rats

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Abstract □ Hemodynamic alterations were studied to determine their role in isoproterenol-induced cardiac arrhythmias in the desoxycorticosterone acetate–saline-treated rat. Since epinephrine, a catecholamine possessing an α-adrenergic receptor agonist component, was considerably less potent as an arrhythmogenic agent, an elevation in blood pressure was thought to be protective against arrhythmias. Both albuterol, a β_2-adrenergic agonist, alone and epinephrine administered following tolazoline, an α-adrenergic blocking agent, decreased blood pressure to that of isoproterenol but failed to elicit significant arrhythmias. Phenylephrine administered prior to isoproterenol resulted in significant arrhythmias despite the maintenance of mean blood pressure at normal levels. The study shows that blood pressure alterations are not important in the etiology of isoproterenol-induced arrhythmias in the corticoid-pretreated rat.

Keyphrases □ Cardiac arrhythmias—isoproterenol induced, effect of hemodynamic alterations, rats treated with desoxycorticosterone acetate □ Isoproterenol-induced cardiac arrhythmias—effect of hemodynamic alterations in rats treated with desoxycorticosterone acetate □ Desoxycorticosterone acetate—pretreatment, effect of hemodynamic alterations on isoproterenol-induced cardiac arrhythmias

Previous research reported that low, normally nontoxic doses of isoproterenol administered to desoxycorticosterone acetate–saline-pretreated rats resulted in severe cardiac arrhythmias, frequently causing death by ventricular fibrillation within 15 min (1). The extraordinary potentiation of acute isoproterenol toxicity (20,000 times) induced by corticoid treatment in animals also was thought to be related to isoproterenol aerosol deaths in asthmatic patients (2). This relationship was made since most patients were severe asthmatics concurrently on long-term prednisone therapy and isoproterenol. This theory was further supported by findings that showed that the fluorocarbon aerosol propellant at levels found in these aerosol products was not arrhythmogenic in the asthmatic patient (3).

BACKGROUND

Many theories were suggested to explain the arrhythmogenic action of isoproterenol in corticoid-treated rats (4–6). The most interesting involved the synergistic alteration of myocardial electrolytes, involving depletion of potassium and magnesium and a gain of sodium associated with isoproterenol and desoxycorticosterone (7). These effects were thought to be associated with the inhibition of membrane function and an arrhythmogenic liability.

The role of blood pressure variations in cardiac rhythm disturbances was studied extensively in hydrocarbon–catecholamine-induced arrhythmias. Although arrhythmogenicity of epinephrine was not related to the height of its blood pressure elevation, arrhythmias disappeared when blood pressure was reduced (8). Intracardiac tension was thought to favor the development of ventricular arrhythmias and ventricular fibrillation. An adrenolytic substituted benzodioxane reversed epinephrine's pressor response and prevented ventricular fibrillation (9). An elevation of blood pressure might be involved in epinephrine's arrhythmogenic action.

Further studies (10, 11) showed that large doses of tolazoline and dibenamine, beyond those necessary to reversal, were able to protect against cyclopropane–epinephrine arrhythmias but that the artificial elevation of blood pressure by using a regulator or compression of the aorta caused a reappearance of these arrhythmias. Normal sinus rhythm and multifocal ventricular tachycardia were induced by varying mechanically the systemic blood pressure during constant epinephrine infusions, but ventricular fibrillation could not be induced (12).

Several studies (13, 14) showed that elevation of blood pressure by 50–100 mm Hg above normal by using a dextran infusion or narrowing the aorta did not influence the fibrillation threshold in the normal anesthetized cat. However, the production of hypertension by the same procedure in the failing heart did decrease the fibrillation threshold. By using cyclopropane–isoproterenol-induced arrhythmias, the effect of blood pressure variation on the arrhythmogenic threshold dose of this catecholamine was studied (15). Changes in systemic blood pressure (decreased from 30 to 60 mm Hg by bleeding or increased 30 mm Hg or more by aortic constriction) had no effect on the arrhythmogenic threshold dose of isoproterenol, which indicates that blood pressure was not an important factor in these arrhythmias.

In addition, bilateral vagotomy or the minimum blocking dose of atropine did not protect against arrhythmias in epinephrine–cyclopropane arrhythmias, nor did bilateral vagotomy alter the effect of aortic occlusion in inducing irregularities after small protective doses of dibenamine (11). Dawes (16) and Katz (17) agreed that blood pressure is not a crucial factor in hydrocarbon–catecholamine-induced arrhythmias and that arrhythmias often are independent of blood pressure variations. Nevertheless, these changes are among the many contributory factors in the production of cardiac arrhythmias.

It was suggested (18) that blood pressure lowering by isoproterenol may decrease coronary flow and that the cardiac stimulatory effects of isoproterenol greatly increase the oxygen requirements of the myocardial fibers. Increased oxygen requirements, together with a decreased coronary flow, lead to hypoxia and thereby myocardial damage. Although hypoxia may be important in the initiation of cellular damage and death, there is controversy as to whether hypoxia is important in the production of arrhythmias.

Szekeres and Papp (19) found a decrease in the fibrillation threshold of cats and dogs administered an oxygen (5–10%)–nitrogen (90–95%) mixture. Turnbull *et al.* (20), however, found no effect on the fibrillation threshold in dogs that had inhaled gas mixtures of various oxygen content, including those used by Szekeres and Papp (19).

The present study was undertaken to evaluate further the importance of hemodynamic alterations associated with isoproterenol in corticoid–isoproterenol arrhythmias.

EXPERIMENTAL

Male albino rats (362) of the Wistar strain, 250–350 g, were divided into four groups. Animals in Group A (desoxycorticosterone acetate–saline) were implanted subcutaneously with a 25-mg desoxycorticosterone acetate pellet in the axillary region while under tribromoethanol anesthesia (25 mg/100 g ip) and were maintained thereafter on 1% saline. Animals in Group B (desoxycorticosterone acetate) were implanted with the same dose of desoxycorticosterone acetate but were maintained on tap water instead of saline. Animals in Group C (saline rats) were maintained on 1% saline instead of tap water. Group D (normal), untreated rats were maintained on tap water and were control animals. All rats had free access to drinking fluid and standard laboratory chow.

At predetermined intervals within 14–20 days, individual rats from these four groups were anesthetized with allobarbital–urethan (0.7 ml/kg ip) and prepared for the recording of blood pressure and ECG. All animals were ventilated artificially with a small respirator. Blood pressure was measured from the left carotid artery with a transducer. The standard limb leads on the ECG were recorded continually on a magnetic tape system. The chest lead selected was obtained by inserting an 18-gauge needle subcutaneously to the left of the sternum so that its tip reached the third intercostal space.

Blood pressure and ECG tracings were monitored on the oscilloscope

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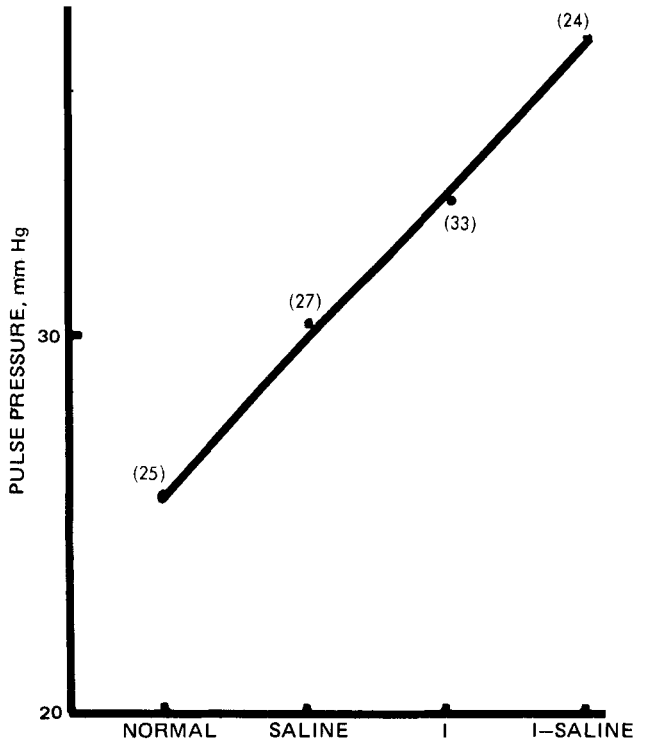

Figure 1—*Pulse pressures associated with the various animal groups.*

screen of a recorder and inscribed intermittently on photorecording paper. Following a 20–30-min stabilization, a single dose of isoproterenol hydrochloride, 10–1000 μg/kg, was injected subcutaneously; each animal was monitored continuously for 30 min and intermittently thereafter for a maximum of 1 or 2 hr.

To record the ECG in conscious, unrestrained rats, 16 desoxycorticosterone acetate–saline rats were prepared several days prior to the experiment in the following manner. Under tribromoethanol anesthesia, fine polytef-coated silver wires were passed under the skin by means of a trochar from each limb to the base of the neck. There the four wires were fixed by a small plaster of Paris cast sutured to the skin in such a manner that long protruding ends of the very flexible wire could be connected to the recording system without substantial restriction or discomfort to the animal. The polytef insulation had been removed from the limb end of the wires, and these terminals had been coated with silver–silver chloride. Statistical significance was determined using the Student *t* test. All animal groups were statistically compared to Group D (normal) rats.

RESULTS

Effect of Treatments—The various animal groups, normal, saline, desoxycorticosterone acetate, and desoxycorticosterone acetate–saline, showed little changes in resting mean blood pressure and heart rate. However, in the desoxycorticosterone acetate–saline group, pulse pressure was increased to the highest extent of all the treatment regimens (Fig. 1).

Effect of Isoproterenol on ECG—The injection of isoproterenol in normal and saline rats did not produce significant ECG changes. In desoxycorticosterone acetate and desoxycorticosterone acetate–saline rats, isoproterenol elicited pronounced ECG alterations and ventricular arrhythmias exemplified by S-T junctional changes, P-R interval prolongation, and higher degrees of A-V block, atrial flutter, and fibrillation.

Ventricular arrhythmias included multifocal premature contractions, coupled ectopic beats (primarily bigemini), ventricular tachycardia, and ventricular fibrillation, which was usually preceded by a short run of ventricular tachycardia or by a single extrasystole. Table I shows that the incidence of ventricular fibrillation was directly proportional to the dose of isoproterenol, reaching a maximum at 150 μg/kg. The comparative incidence of arrhythmias produced by isoproterenol in the four experimental groups showed that desoxycorticosterone acetate (I) animals were far more sensitive to isoproterenol than normal and saline groups, while

Table I—Effect of Isoproterenol on ECG, Heart Rate, and Mean Blood Pressure in the Normal, Saline, I, and I-Saline Rats

Isoproterenol Dose, μg/kg	Treatment	*n*	Arrhythmias		Heart Rate, Beats/min		Blood Pressure	
			Vent.[a]	Fib.[b]	Control, Mean ± *SE*	After Isoproterenol, Mean ± *SE*	Control, Mean ± *SE*	After Isoproterenol, Mean ± *SE*
100	Normal	10	0	0	371.8 ± 11.0	397.2 ± 9.9	122.1 ± 5.2	63.0 ± 4.9[c]
100	Saline	24	2	1	392.1 ± 7.6	415.8 ± 7.3[c]	138.3 ± 3.0	66.3 ± 2.9[c]
100	I	12	7	3	374.3 ± 8.6	407.1 ± 10.6[c]	123.6 ± 5.4	54.7 ± 4.1[c]
100	I–saline	12	12	9	375.4 ± 10.0	402.0 ± 12.0	111.7 ± 0.7	56.4 ± 4.4[c]
150	Normal	10	1	0	359.5 ± 10.3	399.1 ± 9.8[c]	128.8 ± 5.4	57.8 ± 2.8[c]
150	Saline	17	0	0	413.2 ± 7.0	447.8 ± 6.7[c]	145.4 ± 4.4	66.7 ± 2.8[c]
150	I	18	7	5	384.7 ± 6.7	423.8 ± 4.9[c]	134.8 ± 3.4	56.3 ± 2.3[c]
150	I–saline	33	33	26	382.1 ± 6.7	425.1 ± 4.5[c]	125.5 ± 5.6	60.1 ± 3.3[c]
300	Normal	9	1	0	382.2 ± 12.2	414.7 ± 11.2	123.4 ± 5.9	53.8 ± 3.5[c]
300	Saline	17	2	0	397.2 ± 7.9	432.0 ± 7.1	134.0 ± 4.3	60.5 ± 3.3[c]
300	I	11	7	1	401.2 ± 7.3	425.1 ± 7.7	146.0 ± 5.5	62.5 ± 5.1[c]
300	I–saline	12	12	8	378.4 ± 14.2	408.6 ± 12.9	117.7 ± 3.0	45.8 ± 2.2[c]

[a] Ventricular arrhythmias. [b] Ventricular fibrillation. [c] $p < 0.05$.

Table II—Effect of Isoproterenol on ECG, Heart Rate, and Blood Pressure in I–Saline Rats

Isoproterenol Dose, μg/kg	n	Arrhythmias		Heart Rate, Beats/min		Blood Pressure	
		Vent.[a]	Fib.[b]	Control, Mean ± SE	After Isoproterenol, Mean ± SE	Control, Mean ± SE	After Isoproterenol, Mean ± SE
10	9	2	1	373.9 ± 12.0	388.4 ± 13.5	132.4 ± 4.5	77.3 ± 4.4[c]
30	11	8	4	363.9 ± 10.3	381.8 ± 10.6	118.8 ± 6.9	64.0 ± 7.1[c]
60	11	10	5	383.3 ± 12.5	402.7 ± 13.3	131.1 ± 8.8	60.5 ± 6.4[c]
100	12	12	9	375.4 ± 10.0	402.0 ± 12.0	111.7 ± 10.6	56.4 ± 4.4[c]
150	33	33	26	382.1 ± 6.7	425.1 ± 4.5[c]	125.5 ± 5.6	60.1 ± 3.3[c]
300	12	12	8	378.4 ± 14.2	408.6 ± 12.9	117.7 ± 3.0	45.8 ± 2.2[c]
400	14	11	6	366.0 ± 11.5	394.3 ± 12.1	128.1 ± 4.5	55.5 ± 5.3[c]
1000	12	9	3	376.6 ± 7.9	420.4 ± 10.3[c]	130.5 ± 6.0	52.3 ± 4.2[c]
				Unanesthetized			
10	5	5	1	359.2 ± 24.9	536.2 ± 19.0		
30	5	5	2	359.0 ± 9.2	536.2 ± 11.2		
60	4	4	4	396.0 ± 32.3	533.5 ± 5.9		
150	2	2	2	373.0 ± 7.1	518.0 ± 18.0		

[a] Ventricular arrhythmias. [b] Ventricular fibrillation. [c] $p < 0.05$.

Table III—Effect of Adrenergic Agents on the ECG, Heart Rate, and Mean Blood Pressure in I–Saline Rats

Treatment	Dose, mg/kg	n	Arrhythmias		Heart Rate, Beats/min			Blood Pressure, mm Hg		
			Vent.[a]	Fib.[b]	Control[c], Mean ± SE	Control[d], Mean ± SE	Maximum Effect, Mean ± SE	Control[c], Mean ± SE	Control[d], Mean ± SE	Maximum Effect, Mean ± SE
Isoproterenol	0.150	33	33	26	382.1 ± 6.7	—	425.1 ± 4.5	125.5 ± 5.6	—	60.1 ± 3.3[e]
Isoproterenol	0.130	12	12	8	378.4 ± 14.2	—	408.6 ± 12.9	117.7 ± 3.0	—	50.3 ± 8.8[e]
Epinephrine	0.202	6	0	0	408.4 ± 10.0	—	414.2 ± 9.6	134.7 ± 7.5	—	152.0 ± 5.7[e]
Levarterenol	0.125	4	0	0	386.5 ± 15.2	—	418.5 ± 9.4	128.7 ± 9.4	—	181.2 ± 13.9[e]
Albuterol	0.156	5	1	0	409.2 ± 24.9	—	413.0 ± 20.1	133.0 ± 8.5	—	68.8 ± 5.4[e]
Albuterol	0.312	4	0	0	354.8 ± 9.4	—	356.2 ± 6.2	126.0 ± 4.1	—	62.8 ± 1.4[e]
Albuterol	1.560	5	2	1	432.6 ± 20.9	—	451.0 ± 21.2	144.0 ± 11.1	—	78.4 ± 5.4[e]
Tolazoline	8.500	5	5	4	345.8 ± 13.0	368.4 ± 15.4	382.2 ± 15.2	136.6 ± 6.9	123.6 ± 5.7	53.2 ± 4.7[e]
Isoproterenol	0.150									
Tolazoline	8.500	5	1	0	449.4 ± 5.8	425.8 ± 2.6	433.8 ± 6.1	133.6 ± 7.7	105.6 ± 13.5[e]	71.8 ± 7.2[e]
Epinephrine	0.202									
Tolazoline	8.500	5	0	0	400.2 ± 11.4	398.6 ± 9.1	401.8 ± 7.8	149.2 ± 9.4	137.2 ± 8.5	103.8 ± 14.6[e]
Epinephrine	1.360									
Tolazoline	8.500	5	1	0	394.6 ± 9.1	394.4 ± 8.9	412.4 ± 7.8	141.6 ± 9.2	126.4 ± 4.0	140.6 ± 4.4[e]
Levarterenol	0.125									
Tolazoline	8.500	5	0	0	387.2 ± 10.1	396.8 ± 14.7	399.4 ± 10.6	134.8 ± 7.8	119.2 ± 7.4	95.4 ± 10.3[e]
Epinephrine	0.202									
Levarterenol	0.125									
Phenylephrine	1.500	5	5	5	414.8 ± 7.2	407.6 ± 11.5	427.0 ± 2.4	136.2 ± 5.3	175.4 ± 10.0[e]	120.0 ± 13.0[e]
Isoproterenol	0.150									
Phenylephrine	3.000	5	5	3	421.2 ± 16.0	413.2 ± 10.2	441.4 ± 12.8	122.0 ± 4.1	177.0 ± 7.3[e]	105.0 ± 4.5[e]
Isoproterenol	0.150									

[a] Ventricular arrhythmias. [b] Ventricular fibrillation. [c] No drug treatment. [d] Following first drug treatment. [e] $p < 0.05$.

desoxycorticosterone acetate (I)–saline animals were most sensitive to isoproterenol, with a majority of the animals experiencing ventricular fibrillation (Table I).

Effect of Isoproterenol on Heart Rate—The maximum chronotropic effect of various doses of isoproterenol in anesthetized desoxycorticosterone acetate (I) rats is illustrated in Table II. No significant difference was encountered in the control heart rate of either group, but isoproterenol induced an increase in heart rate in anesthetized desoxycorticosterone acetate (I)–saline rats at all doses. However, a significant difference from control animals could only be detected at 150 and 100 μg/kg and at all dosages in unanesthetized animals.

Effect of Isoproterenol on Blood Pressure—The data presented demonstrate that 100, 150, and 300 μg of isoproterenol/kg in all treatment groups produced a significant fall in blood pressure (Table I). When increasing dosages of isoproterenol were administered to desoxycorticosterone acetate (I)–saline rats, a significant consistent fall in blood pressure was observed at all doses (Table II).

Substitution of Epinephrine for Isoproterenol—Epinephrine bitartrate was employed instead of isoproterenol to determine whether another catecholamine possessing a potent β-adrenergic agonist component would produce arrhythmias in desoxycorticosterone acetate–saline rats. Under allobarbital–urethan anesthesia, no arrhythmias were elicited in rats treated with 202 μg of epinephrine/kg (equivalent to 150 μg of isoproterenol/kg) or in an additional group of animals treated with repeated doses of epinephrine up to a total of 4.0 mg/kg.

Since epinephrine is both an α- and a β-adrenergic receptor agonist, 10 desoxycorticosterone acetate–saline-pretreated, anesthetized rats were injected subcutaneously with the α-receptor blocking agent tolazoline (8.5 mg/kg). Ten minutes later, half of these animals were injected subcutaneously with 202 μg/kg; the remainder received 1.36-mg/kg doses of epinephrine. By this technique, it was expected that the unopposed β-adrenergic activity of epinephrine might elicit arrhythmias. However, essentially no cardiac irregularities were encountered, although both doses of epinephrine elicited hypotension instead of hypertension, which indicates effective blockade of vascular α-receptors by tolazoline (Table III). The same tolazoline pretreatment did not prevent ventricular arrhythmias and death in fibrillation in an additional group of desoxycorticosterone acetate (I)–saline rats injected with 150 μg of isoproterenol/kg (Table III).

A similar experiment was carried out in 40 conscious unrestrained rats pretreated for 3 weeks with desoxycorticosterone acetate–saline, using identical dosages of tolazoline and epinephrine. Fourteen animals in this group died within 1 hr in ventricular fibrillation. Thus, on the weight basis, epinephrine appeared to possess a weaker arrhythmogenic potential than isoproterenol, which became manifest only in the absence of anesthesia.

When levarterenol hydrochloride was substituted for isoproterenol in a dose of 125 μg/kg (equivalent to 150 μg of isoproterenol/kg), it increased heart rate and blood pressure but did not produce ventricular arrhythmias or fibrillation (Table III). When levarterenol was administered in the presence of α-adrenergic blockage (tolazoline, 8.5 mg/kg, administered prior to levarterenol), it likewise produced minor ventricular arrhythmias and no ventricular fibrillation (Table III).

Blood Pressure Alterations by Phenylephrine—Since isoproterenol normally caused a fall in blood pressure, it was of interest to determine whether arrhythmias could be induced by isoproterenol when blood pressure was maintained above 100 mm Hg. Therefore, phenylephrine, an α-adrenergic agonist with minimal cardiac stimulatory effect, was injected, and when the animals were clearly hypertensive (3–5 min), isoproterenol was administered (Table III). In these rats, isoproterenol consistently induced ventricular arrhythmias and ventricular fibrillation, although the mean blood pressure was maintained at normotensive levels of 105 and 120 mm Hg (Table III).

Blood Pressure Alteration with Albuterol—Albuterol, a β_2-agonist with preferential ability to activate vascular and bronchial smooth muscle rather than cardiac muscle, afforded the opportunity to produce hypotension with minor cardiac stimulatory effects. As expected, the administration of albuterol in doses equivalent to 0.15, 0.30, and 1.5 mg of isoproterenol/kg caused a fall in blood pressure comparable to isoproterenol and did not consistently produce arrhythmias or ventricular fibrillation (Table III).

DISCUSSION

It was surprising that epinephrine did not elicit cardiac arrhythmias in the anesthetized desoxycorticosterone acetate–saline rats since the arrhythmogenic properties of this agent closely resembled those of isoproterenol in many standard methods for the production of arrhythmias. This result may indicate the effect of general anesthesia and membrane depressants in protecting against these arrhythmias. Epinephrine also differed from isoproterenol because it inconsistently produced ventricular fibrillation in the conscious desoxycorticosterone acetate–saline animals and only if they had been previously treated with tolazoline. This finding, that epinephrine elicited arrhythmias only after α-adrenergic blockade, points to the importance of β-adrenergic stimulation in the genesis of these arrhythmias. Since α-adrenergic stimulants increase the fibrillation threshold (21), the α-adrenergic component of epinephrine may have exerted an antiarrhythmic action. Therefore, blockade of α-receptors by tolazoline would allow the predominance of β-activation and the emergence of ventricular arrhythmias and fibrillation.

β-Adrenergic stimulation produces, among other effects, myocardial ischemic changes and hypotension. In these experiments, desoxycorticosterone acetate and desoxycorticosterone acetate–saline rats appeared to be more sensitive to the ischemic changes induced by isoproterenol, because the most consistent, earliest, and prominent ECG change observed was a depression of the S-T junction. Myocardial ischemia probably increased the likelihood of rhythm irregularities in these animals because it enhances pacemaker activity (22, 23), slows conduction, and increases the dispersion of recovery of excitability in ventricular muscle (24).

Epinephrine, on the other hand, failed to produce ischemic changes as evidenced by the absence of S-T junctional depression. This difference between isoproterenol and epinephrine may have been due to elevation of blood pressure by the latter, which may have increased coronary perfusion and thus prevented the manifestation of ischemia. It was of interest to determine the role of blood pressure in the etiology of these arrhythmias, and two series of experiments were performed.

In the first experiments, albuterol, an agent more selective toward vascular than cardiac β-receptors (25, 26), produced hypotension comparable to that of isoproterenol without S-T junctional depression and did not elicit ventricular fibrillation. However, with a larger dose of this agent, one instance of ventricular fibrillation was obtained, probably as a consequence of a loss of its β-receptor selectivity. In the second set of experiments, when isoproterenol was administered in the presence of phenylephrine, mean blood pressure did not fall below levels to impair adequate coronary perfusion. Nonetheless, it consistently elicited a depression of the S-T junction as well as ventricular fibrillation. These results led to the same conclusion reached by other investigators: that blood pressure changes do not play a major role in the genesis of rhythm disturbances.

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Nitroglycerin Stability in Polyethylene Glycol 400 and Povidone Solutions

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Abstract □ The stability of solutions of nitroglycerin in several common pharmaceutical solvents and compounds used as tablet excipients was investigated using a UV spectrophotometric assay. Included in the study were povidone (I), polyethylene glycol 400 (II), and solvents such as absolute alcohol, propylene glycol, and glycerol. At the elevated temperatures of the accelerated stability studies, only II demonstrated a considerable adverse effect on the stability of the nitroglycerin solution. It is postulated that a "reaction compound" was formed between nitroglycerin and II which regenerated nitroglycerin depending on hydrolysis conditions. Based on the Arrhenius equation and with initial rates of up to 6 hr of degradation, the predicted stability for a II solution of nitroglycerin in terms of its 10% decomposition at 25° was approximately 7 days.

Keyphrases □ Nitroglycerin—stability in polyethylene glycol 400 and povidone solutions □ Stability—nitroglycerin in polyethylene glycol 400 and povidone solutions □ Polyethylene glycol 400—nitroglycerin solutions, stability □ Povidone—nitroglycerin solutions, stability □ Vasodilators, coronary—nitroglycerin, stability in polyethylene glycol 400 and povidone solutions

The problems encountered with nitroglycerin tablet stability, contributing to a loss of content uniformity (1) and possible potency variation, were the direct result of a measurable vapor pressure of nitroglycerin at room temperature (2). Attempts to overcome these difficulties included changes in the material of the nitroglycerin dosage form container and its cap liner (2). However, nitroglycerin volatilization still was not controlled effectively (3).

Reformulation of the tablet by incorporating "fixatives" (4–7) and changing the manufacturing process from molding to direct compression (8) were other attempts to control this physical problem. These steps were demonstrated to reduce successfully nitroglycerin volatility. For instance, polyethylene glycol 400 (II) (4) and povidone (I) (5, 6) have been employed as fixatives or stabilizing agents in nitroglycerin tablets to reduce volatility and thus maintain content uniformity and potency. A combination of I and microcrystalline cellulose (7), used as stabilizers, was demonstrated to be even more effective (3). The evaluation of these reformulated tablets was based on content uniformity and various physical tests, such as hardness, disintegration, and volatility, as well as exposure to several extreme conditions.

The current study was conducted to investigate the possible chemical interactions between nitroglycerin in solution and I and II since no such data were reported previously. If I or II exerted a detrimental effect, further studies would be conducted to predict nitroglycerin stability in the particular solvent.

EXPERIMENTAL

Nitroglycerin Stock Solution—*Preparation*—A 5-g portion of nitroglycerin mixture powder[1] in lactose was weighed and placed in a suitable separator. Chloroform[2], 10 ml, and 50 ml of deionized distilled water were added and the mixture was shaken gently for about 5 min to extract the nitroglycerin. The lower chloroform layer containing the extracted nitroglycerin was filtered[3].

The chloroform solvent was evaporated by blowing nitrogen gas on the surface until a constant weight of the pale-yellow viscous liquid of nitroglycerin was obtained. A sufficient volume of absolute alcohol was added to the weighed residue to obtain a nitroglycerin stock solution of approximately 1.0 mg/100 μl.

Standardization—This step was based on the USP method (9) with slight modifications. A standard solution of potassium nitrate was prepared by dissolving about 80 mg of potassium nitrate pellets[4], accurately weighed, with 1 ml of deionized distilled water in a 100-ml volumetric flask. Sufficient acetic acid[5] was then added to volume and mixed. A 100-μl volume of nitroglycerin solution to be standardized was withdrawn accurately and placed in a 100-ml volumetric flask containing 1 ml of acetic acid.

After the addition of 2 ml of phenoldisulfonic acid reagent, the mixture was shaken vigorously on a mechanical shaker for 3 min and allowed to stand at room temperature for another 15 min. Approximately 25 ml of deionized distilled water was added to dilute the reaction mixture before the addition of 10 ml of strong ammonia solution[5] and its final dilution to volume with deionized distilled water. Concurrently, 1 ml of the previously prepared standard potassium nitrate solution was pipetted into a 100-ml volumetric flask and treated similarly for the analysis as in the sample.

The absorbances at 410 and 600 nm of both solutions were determined simultaneously against a reagent blank. The strength of the nitroglycerin stock solution was then calculated from the equation:

$$\text{mg}/100\ \mu\text{l} = 0.749C[(A_{410} - A_{600})_U/(A_{410} - A_{600})_S] \quad \text{(Eq. 1)}$$

where 0.749 is a factor for relating the nitrate content of potassium nitrate to that of nitroglycerin, C is the potassium nitrate concentration in milligrams per milliliter in the potassium nitrate standard solution, and the subscripts U and S refer to the nitroglycerin sample and the potassium nitrate standard, respectively.

[1] S.D.M. No. 17, ICI America Inc., Wilmington, Del.
[2] ACS grade, Allied Chemical Corp., Morristown, N.J.
[3] Whatman filter paper No. 2.
[4] NF XI, Matheson, Coleman & Bell, Norwood, Ohio.
[5] ACS grade, Fisher Scientific Co., Fair Lawn, N.J.

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