Evaluation of Caffeine and Ouabain as Permissive Agents in the Production of Myocardial Necrosis

By DUANE G. WENZEL, JOHN D. MACCARTHY, and CHARLES O. RUTLEDGE

Twelve groups of six rabbits per group were treated with nicotine, caffeine, ouabain, hypercholesterolemic diet, and various combinations of these for 24 weeks. Systolic blood pressures and coagulation times were obtained at 4-week intervals, and a histopathological examination of the hearts was made at death or sacrifice. Treatments including either nicotine or cholesterol significantly reduced the coagulation times. Most nicotine-treated groups demonstrated increases in systolic pressures. While myocarditis was observed in several of the animals receiving multiple treatments, such treatments did not increase the incidence or severity of coronary atherosclerosis.

THE CHRONIC oral ingestion of nicotine by hypercholesterolemic rabbits receiving periodic injections of ergonovine has been demonstrated to induce myocardial necrosis (1). Treatment with nicotine alone increases the systolic blood pressure and the rate of blood coagulation (2). Although it is generally accepted that hypertension and hypercoagulability of the blood may be related to myocardial infarction in the human, there has been no correlation between such nicotine-induced changes and myocardial necrosis in the experimental animal. The role of ergonovine in this cardiac-damaging regimen is still speculative. In contrast to other ergot alkaloids, ergonovine is not sympatholytic but actually enhances cardiovascular actions of the catecholamines (3). One might expect that it would similarly affect the catecholamine-mediated actions of nicotine. In fact, the combination of nicotine and ergonovine has been demonstrated to induce thickening and fibrosis of the small coronary branches (1). Such an effect suggests that a damaged vascular bed with the resultant alteration in tissue circulation may predispose the myocardium to pathological changes induced by atherosclerosis. The postulated requirement of pre- or concomitantly existent damage for the production of hypercholesterolemia-induced pathological changes has been suggested by others (4) and is consistent with the usual absence of coronary thrombosis or infarcts in animals receiving a hypercholesterolemic diet as their sole treatment.

In the present problem it was hypothesized that infarcts and/or myocardial necrosis may result when hypercholesterolemia is superimposed upon underlying conditions which damage the myocardium or its circulatory system. To test this hypothesis, caffeine and a cardiac glycoside were employed in an attempt to induce such an underlying condition. It has been demonstrated that the injection of several doses of caffeine to either guinea pigs or rats will induce degenerative changes of the myocardium (5). Similar responses in the rabbit have been reported to result from a single combined dose of caffeine and epinephrine (6), while strophanthin in conjunction with epinephrine has also induced this reaction (7). It was anticipated that the catecholamine-releasing action of nicotine could replace the administration of epinephrine *per se*.

Received June 3, 1963, from the Division of Pharmacology, School of Pharmacy, University of Kansas, Lawrence.
Accepted for publication July 17, 1963.
Supported by a grant from the Life Insurance Medical Research Fund.

EXPERIMENTAL

Seventy-two female, albino, New Zealand strain rabbits were divided at 6 weeks of age into 12 groups of six per group. Each group received one of the following treatments: control, nicotine (N); hypercholesterolemic diet (HCD), N + HCD; caffeine (C), C + N, C + HCD, C + HCD + N; ouabain (O), O + N, O + HCD, O + HCD + N. Control treatment consisted of Purina rabbit chow and water *ad libitum*. Nicotine dosage was the equivalent of one pack of cigarettes daily on the basis of body weight, or 1.14 mg./Kg./day in the drinking water (8). The hypercholesterolemic treatment consisted of the basal diet to which 5% cottonseed oil and 1% cholesterol were added. Caffeine, in a dose of 5 mg./Kg. of the alkaloid, was injected intravenously as the sodium benzoate salt once each week; ouabain, 0.025 mg./Kg., was similarly administered. Each of these were given in one-half milliliter of isotonic saline.

Systolic pressures were determined with a Grant ear capsule at the initiation of treatment and at 4-week intervals thereafter. Coagulation times were obtained by the capillary tube method with blood from a stab wound in the tail. All measurements were performed on the same days that the caffeine and ouabain were administered and always preceded these injections. By this procedure the acute response to the administration of the drugs did not affect the blood pressure and coagulation

TABLE I.—EFFECTS OF NICOTINE, CAFFEINE, OUABAIN, AND HYPERCHOLESTEROLEMIA ON BLOOD PRESSURE AND COAGULATION TIME

Treatment	Difference from Initial Values at 16 Wks.	
	Systolic Pressure, mm. Hg	Coagulation Time, Sec.
Control	2	−1.8
Nicotine	32[a]	−7.9
Cholesterol	0	−4.9
Nicotine + cholesterol	21	−13.2
Caffeine	11	−10.4
Caffeine + nicotine	31[a]	−9.6
Caffeine + cholesterol	2	−10.6
Caffeine + nicotine + cholesterol	9	−12.4[b]
Ouabain	6	−6.2
Ouabain + nicotine	8	−8.9
Ouabain + cholesterol	−5	−10.5
Ouabain + nicotine + cholesterol	19[b]	−12.2[b]

[a] $P < 0.01$. [b] Statistically different from control ($P < 0.05$).

time determinations. Because of the loss of as many as four out of six animals in some groups after 16 weeks, blood pressure and coagulation time determinations were discontinued at this time. Treatments were continued for 24 weeks. The hearts of those animals which died during this period and the survivors which were sacrificed at 24 weeks were examined for microscopic evidence of pathological damage. Cross sections were made perpendicular to the basal apical axis at the level of the aortic ring valve and midway between the aortic ring and apex.

RESULTS AND DISCUSSION

Mean systolic blood pressures and blood coagulation times are presented in Table I. The Dunnett "t" test (9) was performed to determine the significance of differences between the control and test values at 16 weeks. As indicated in Table I, all nicotine-treated groups, except nicotine combined with ouabain or with caffeine and the hypercholesterolemic diet, induced significant ($p < 0.05$ and $p < 0.01$) increases in systolic pressure. There are no obvious reasons for the lack of a significant increase by these two groups. Coagulation times were consistently reduced by all treatments, although only nicotine or the cholesterol treatment—with or without caffeine or ouabain—caused a significant ($p < 0.05$) lowering.

Histopathological examination revealed minimal to advanced subintimal lipid deposition in the small to medium coronary arteries of all animals treated with the cholesterol diet. There was no indication that any of the treatments increased either the severity or the extent of the lipid deposition. Examination of the myocardium disclosed the presence of minimal focal areas of myocarditis in six of the treated animals. Two of these had received ouabain, nicotine, and cholesterol, while two were from the caffeine-nicotine-cholesterol group. One animal had received ouabain and another caffeine and nicotine. While the lack of more severe and consistent lesions does not allow any clear cut conclusions, it is apparent that those animals receiving the multiple treatment accounted for more than their anticipated share of the myocarditis. As there was no evidence of necrosis in the hearts, it is also apparent that the original hypothesis stating that myocarditis predisposes to atherosclerotic damage was not substantiated. It should be recognized that there was no real evidence that the injections of ouabain or caffeine even produced myocarditis. It is suggested that either the caffeine or ouabain treatments were not sufficiently effective to induce myocarditis or after repeated injections that the tissue reaction was reduced.

REFERENCES

(1) Wenzel, D. G., Turner, J. A., Jordan, S. W., and Singh, J., *Circulation Res.*, **9**, 694(1961).
(2) Wenzel, D. G., Singh, J. K., and Turner, J. A., *Ann. N. Y. Acad. Sci.*, **90**, 302(1960).
(3) Wills, J. H., *Federation Proc.*, **10**, 148(1951).
(4) Katz, L. N., and Stamler, J., "Experimental Atherosclerosis," Charles C Thomas, Springfield, Ill., 1953, p. 229.
(5) Mehes, G., and Szekeres H., *Acta Physiol. Acad. Sci. Hung.*, **6**, 113(1954).
(6) Fleisher, M. S., and Loeb, L., *Arch. Internal Med.*, **3**, 78 (1909).
(7) Sollmann, T. E., "A Manual of Pharmacology," W. B. Saunders Co., Philadelphia, Pa., 1957, p. 490.
(8) Wenzel, D. G., and Beckloff, G. L., THIS JOURNAL, **47**, 338(1958).
(9) Dunnett, C. W., *J. Am. Statistical Assoc.*, **50**, 1096 (1955).

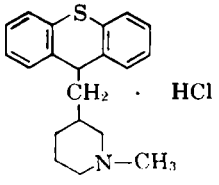
Antispasmodic Effects of 9-[(*N*-Methyl-3-piperidyl)methyl] Thioxanthene Hydrochloride

By H. LAUENER* and R. C. POGGE

A new compound, 9-[(*N*-methyl-3-piperidyl)methyl] thioxanthene hydrochloride (methixene hydrochloride)[1] has shown parasympatholytic properties, strong inhibition of gastrointestinal motility in rats, mice, and guinea pigs and less active inhibition of salivation and pupil dilation. Its oral toxicity is low.

CAVIEZEL, ET AL., have described the synthesis (1) and pharmacology (2) of a large series of thioxanthene derivatives. During early studies, interest was concentrated on the antitremorine properties which resulted in trial of one of these compounds—subsequently identified as methixene hydrochloride—in the symptomatic treatment of paralysis agitans for which relatively high dosage is required. The early work included mention of inhibition of gastrointestinal motility even by low doses of 9-[(*N*-methyl-3-piperidyl)methyl] thioxanthene hydrochloride. Later, clinical investigators explored the possibility that this compound might be useful in the symptomatic treatment of manifestations of gastrointestinal hypermotility and spasm. The clinical findings will be published elsewhere (3–5). The present study represents an expansion of the preliminary work to include motility studies in three species of experimental animals.

The chemical structure of methixene hydrochloride may be represented



EXPERIMENTAL

Inhibition of Intestinal Passage of Charcoal.—The action of methixene hydrochloride upon the

Received April 2, 1963, from Dorsey Laboratories, Lincoln, Nebr.
Accepted for publication July 19, 1963.
* Present address: Research Institute of Dr. A. Wander S. A., Berne, Switzerland.
[1] Marketed as Trest by Dorsey Laboratories, Lincoln, Nebr.