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.

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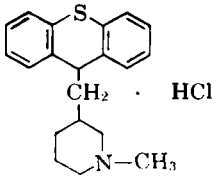
Antispasmodic Effects of 9-[(*N*-Methyl-3-piperidyl)methyl] Thioxanthene Hydrochloride

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

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[1] Marketed as Trest by Dorsey Laboratories, Lincoln, Nebr.

rate of intestinal passage of charcoal in mice and rats was studied at the Wander Research Institute, Berne, Switzerland. The method employed was a modification of that reported originally by Macht, *et al.* (6). Specifically, the time during which a standard dose of atropine shows an optimum inhibitory effect was selected as the interval to be observed between administration of charcoal and sacrificing of the animals.

Male rats and mice of each sex were used. The animals were deprived of food on the previous evening. Five minutes after intravenous injection of either methixene hydrochloride or atropine, a charcoal suspension containing 1 Gm. of active charcoal (Norit A) and approximately 100 mg. of gum arabic in 7 ml. of distilled water were administered by stomach tube. Mice and rats were sacrificed by concussion 10 and 30 minutes later, respectively; the entire small intestine was quickly extirpated.

The distance traveled by the charcoal suspension and the total length of small intestine were measured with the intestine hanging freely.

In all animals, the distance traveled by the charcoal suspension was calculated in per cent of bowel length. Mean values of the treated groups were expressed in percentage of the control group. From the dose-effect straight line graphs, it was possible to read off the ED_{50}, *i.e.*, the dose with which the transport of charcoal would be delayed by 50%. Important data on the method are shown in Table I.

TABLE I.—METHOD USED IN DETERMINING THE ACTION OF METHIXENE HYDROCHLORIDE ON MICE AND RATS

	Mice	Rats
Animal weight	18–21 Gm.	140–180 Gm.
Animals per dose	10	6
Interval between administration of charcoal and sacrificing	10 min.	30 min.
Volume of charcoal suspension	0.05 ml.	0.2 ml.
Mean length of small intestine	45 cm.	100 cm.
Distance traveled by charcoal in per cent of bowel length in control animals	40–60%	55–75%

Action on Reflex Peristalsis in the Anesthetized Guinea Pig.—Guinea pigs (weighing 300–400 Gm.) which had been fasted for 24 hours were anesthetized with 1.2 Gm./Kg. of urethane administered subcutaneously. The intestine was ligated about 10 cm. from the cecal end of the ileum and a cannula tied about 6 cm. craniad. The cannula was connected to a pressure-recording instrument (pneumatic pressure-intensifier) and a water storage vessel which made possible a continuous raising of the pressure in the separated ileum segment. The peristaltic pressure-waves beginning with a constant pressure for each animal were measured. It has been found in our experiments that following injection of parasympatholytic agents, the critical pressure threshold does not rise, but the intensity and duration of the peristaltic waves decrease.

The peristaltic reflex was induced at intervals of 10 minutes by increasing the pressure for 3 minutes, then lowering it to normal. Thirty minutes after the operation, it was generally possible to reproduce the number and intensity of the pressure waves. To evaluate the action of the preparations, the wavelike increases of pressure for every 10 minutes of experimental period were added. The mean of three or four values before injection of the preparations was calculated; the values just after intravenous injection of the preparations were expressed in percentage of the control mean.

It was possible to read off the ED_{50}, *i.e.*, the dose with which reflex peristalsis would be inhibited by 50%, from the dose-effect curve. The preparations were injected intravenously in doses increasing threefold, the next higher dose being injected only when the peristaltic reflex could be induced in repeated experiments to the same extent.

Inhibition of Salivation in the Mouse and Rat.—Salivation was induced by intraperitoneal injection of 1 mg./Kg. of pilocarpine. At the same time the test preparation was injected intravenously (five rats or ten mice per dose). Salivation was observed 15 and 30 minutes after injection by wiping the mouth of the animal on a blotting paper. Only the presence or absence of saliva was noted. It was possible to draw a dose-effect curve with the number of animals without saliva in percentage of the number per group and to determine the ED_{50} from it.

Inhibition of Salivation in the Guinea Pig.—Salivation was induced by intraperitoneal injection of 3 mg./Kg. of pilocarpine. The test preparation

TABLE II.—PHARMACOLOGIC RESULTS OF METHIXENE HYDROCHLORIDE AND ATROPINE

	Methixene Hydrochloride	Atropine	Ratio Methixene Hydrochloride/ Atropine
Effect on Gastrointestinal Motility			
Mouse: ED_{50}, mg./Kg. i.v. for inhibition of charcoal passage	6.8	2.9	2.2:1
Rat: ED_{50}, mg./Kg. i.v. for inhibition of charcoal passage	1.4	0.33	4.2:1
Guinea Pig: ED_{50}, mg./Kg. i.v. for inhibition of peristaltic reflex	0.15	0.009	16.7:1
Effect on Salivation			
Mouse: ED_{50}, mg./Kg. i.v. (50% inhibition)	3.2	0.1	32:1
Rat: ED_{50}, mg./Kg. i.v. (50% inhibition)	3.5	0.04	87:1
Guinea Pig: ED_{50}, mg./Kg. i.v. (50% inhibition)	4.0	0.062	64.5:1
Mydriatic Effect			
Mouse: ED_{300}, mg./Kg. s.c.	1.3	0.065	20:1

was injected simultaneously and also intraperitoneally (eight animals per dose). Salivation was observed 30 minutes after injection by wiping the mouth of the animal on a blotting paper; the ED_{50} was determined by the same procedure employed in the experiment on mice and rats.

Action on Pupillary Width.—The pupillary width in unanesthetized mice was measured with a binocular lens with a magnification of 15 times 30 minutes after subcutaneous administration of the drugs (Pulewka, P., *Arch. Exptl. Pathol. Pharmakol.*, **168**, 307(1932)). The ED_{300} (dose which increases the pupil size to 300% of the control value) was determined.

RESULTS

Pharmacologic results of methixene hydrochloride and atropine are shown in Table II.

In three species tested, the relative potency of methixene hydrochloride to atropine reveals that with respect to inhibition of gastrointestinal motility, atropine is 2.2 to 16.7 times as potent, but with reference to inhibition of salivation, atropine is 32 to 87 times as potent. In the mouse, atropine is 20 times as potent as methixene hydrochloride with respect to mydriatic effect.

ACUTE TOXICITY

The values of the LD_{50} and their confidence limits have been calculated according to the Litchfield-Wilcoxon method (7). The intravenous LD_{50} for methixene hydrochloride is 18.0 (15.8 to 20.5) mg./Kg. in mice and 24.0 (21.0 to 27.4) mg./Kg. in rats. Orally, the LD_{50} is 430 (350–530) mg./Kg. in mice and over 1,500 mg./Kg. in rats.

CHRONIC TOXICITY

Initial studies on the tolerance of methixene hydrochloride during long term administration were carried out in rats. Three groups of ten animals each received 1, 4, and 15 mg./Kg. of the drug with the food for 9 months. An untreated group served as the controls. Weight gain in the treated animals and controls, hematological findings, and macroscopic and histologic examination of the organs gave no indication of toxic action of methixene hydrochloride in this dosage (2). Studies in rats for 18 months and in dogs for 12 months and suitable progeny studies are in progress and will be reported when completed.

CONCLUSION

It is evident that methixene hydrochloride is somewhat less active than atropine in the intestinal passage test and also with regard to inhibition of the peristaltic reflex. However, if the characteristic secondary actions of parasympatholytic agents, inhibition of salivation, and mydriatic action are considered, far higher doses of methixene hydrochloride are required to produce these effects than those of atropine.

In the three animal species studied, the therapeutic ratio of methixene hydrochloride (range between gastrointestinal effect and undesired side effects) is from four to 21 times higher than that of atropine.

Studies in experimental animals justify the therapeutic evaluation of methixene hydrochloride in the symptomatic management of conditions associated with gastrointestinal hypermotility or spasm.

Long term toxicity studies and progeny studies should be completed before methixene hydrochloride is released for general use.

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Analysis of Dosage Forms Containing Ephedrine and Barbiturate Combinations

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Procedures are presented for the determination of ephedrine salts in combination with barbiturates in tablets and capsules. An aliquot prepared from the dosage form is passed through a strong anion exchange resin. The ephedrine, contained in the eluate, is determined by titration with standard hydrochloric acid. The barbiturate is eluted from the column with acetic acid in ethanol and determined by nonaqueous titration. A modification of this procedure is proposed for formulations containing the sodium salt of the barbiturate. The methods are simple, accurate, and less time consuming than the official assay.

THE OFFICIAL ASSAY (1) for ephedrine sulfate and phenobarbital capsules involves ether extraction of the phenobarbital and a Kjeldahl distillation procedure for the estimation of the ephedrine content. The assay procedure has remained essentially unchanged since N.F. VIII when this dosage form first became official. The distillation technique was originally proposed by Hilty (2). The ephedrine sulfate solution is refluxed with hydrochloric acid and distilled in the presence of zinc dust and sodium hydroxide into a solution of standard acid. The ephedrine is determined by residual titration of the distillate. The method is tedious and time consuming.

Hilty and Wilson (3) analyzed tablets containing a combination of ephedrine sulfate and a barbiturate

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