Antagonism of *dextro*-Propoxyphene Poisoning in Albino Mice with Nalorphine HCl, Levallorphan Tartrate, and Methylene Blue

By HOWARD S. HARPEL, JR., and DAVID E. MANN, JR.

The intraperitoneal administration of *d*-propoxyphene HCl to albino mice at a dosage level of 200 mg./Kg. was lethal to all 80 controls. The prophylactic subcutaneous administration of either nalorphine HCl, levallorphan tartrate, or methylene blue produced a significant increase in the number of survivors and also increased significantly the mean survival time of the remainder. Methylene blue in combination with nalorphine HCl potentiated the protective effect of nalorphine HCl, while the dye in combination with levallorphan tartrate reduced the antidotal effectiveness of the latter. Neither malachite green oxalate nor quinacrine dihydrochloride was able to produce significant protection against lethal doses of *d*-propoxyphene.

THE PHARMACOLOGICAL properties of *α-dl*-4-dimethylamino - 1,2 - diphenyl - 3 - methyl-2-propionyloxybutane (*dl*-propoxyphene) were first described in 1955 by Robbins, who reported that oral and subcutaneous doses of this compound produced a profound morphine-type analgesia in rats and dogs without the development of tolerance (1). After resolution of the optically active isomers, the *dextro* isomer (*d*-propoxyphene) exhibited twice the analgesic potency of the isomeric mixture on a weight basis, while the *levo* isomer was ineffective (2).

Clinically, *d*-propoxyphene was shown to produce effective analgesia similar to that of codeine when administered orally as the hydrochloride salt alone or in combination with acetophenetidin, acetylsalicylic acid, and caffeine (3–5).

In March 1960, the National Clearinghouse for Poison Control Centers reported a total of 12 cases of accidental ingestion and acute overdosage of *d*-propoxyphene (6). Since that time, a number of additional episodes have appeared in the literature (7–9). Even with quantities many times greater than the therapeutic dose, relatively rapid recoveries were made if gastric lavage and symptomatic therapy were instituted before the onset of coma or convulsions. A more serious clinical picture occurred with the ingestion of extremely large amounts or a delay in the initiation of symptomatic therapy.

There is no specific antidote for *d*-propoxyphene poisoning (10). Except for one fatal episode, symptomatic supportive therapy appeared to be responsible for the recovery of all of the patients whose cases are reported in the literature. Convulsions were controllable by the intravenous injection of pentobarbital (8), deep intramuscular injection of paraldehyde, or inhalation of halothane (10); however, extreme caution is necessary to avoid the enhancement of depression. Respiration was maintained by tracheotomy and artificial respiration, and the administration of caffeine and sodium benzoate has been reported to be of some value (7).

Nalorphine HCl and levallorphan tartrate have been used in several cases, although their clinical value in this situation is still doubtful (8). Their effects are not so rapid or dramatic as against the more potent narcotic analgesics.

Acute lethal doses of *d*-propoxyphene in common laboratory animals are many times higher than those necessary to produce effective analgesia. Robbins (1) first reported some success in antagonizing isomeric propoxyphene toxicity in rats with nalorphine HCl. Subsequently, Chapman and Walaszek (11) reported that the administration of 0.5 mg./Kg. of nalorphine HCl prior to *d*-propoxyphene raised the LD_{50} of the analgesic from 68 to 105 mg./Kg.; but even though some improvement was noted, convulsions produced by *d*-propoxyphene were only partially blocked.

The purpose of this investigation was to evaluate the effectiveness of nalorphine HCl, levallorphan tartrate, methylene blue, malachite green oxalate, and quinacrine dihydrochloride in protecting mice against a lethal dose of *d*-propoxyphene.

EXPERIMENTAL

Adult male albino mice (Huntingdon Farms, HTF strain), weighing between 20 and 25 Gm., were

Received August 26, 1964, from the Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, Pa.

Accepted for publication October 1, 1964.

Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.

Abstracted from a thesis presented by Howard S. Harpel, Jr., to the Graduate School, School of Pharmacy, Temple University, in partial fulfillment of Master of Science degree requirements.

The authors are indebted to Dr. J. M. McGuire, Eli Lilly and Co., Indianapolis, Ind., for supplying *d*-propoxyphene HCl (Darvon) and to Dr. A. Schribner, Winthrop Laboratories, Rensselaer, N. Y., for supplying quinacrine (Atabrine).

TABLE I.—EFFECT OF SUBCUTANEOUS PRETREATMENT WITH VARIOUS AGENTS UPON THE TIME AND RATE OF SURVIVAL FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF 200 MG./KG. OF *d*-PROPOXYPHENE HCl

Drug	Dose, mg.	Time Prior to *d*-Propoxyphene, Min.	Animals, No.	Mean Survival Time, Sec.[a]	Increase in Mean Survival Time, Sec.	Survivors, No.	Survival, %
Control	...	..	80	330	...	..	...
Methylene blue	0.1	5	40	412	82	2	5.0
Methylene blue	0.1	10	40	420	90	2	5.0
Methylene blue	1.0	5	39	345	15[c]	3	7.7
Methylene blue	1.0	20	39	341	11[c]	2	5.1
Methylene blue[b]	1.0	30	37	441	111	3	8.1
Nalorphine HCl	0.1	5	38	586	256	7	18.4
Levallorphan tartrate	0.25	5	38	510	180	8	21.1
Nalorphine HCl and Methylene blue	0.1 and 0.1	5	36	642	312	11	30.6
Levallorphan tartrate and Methylene blue	0.25 and 0.1	5	40	482	152	2	5.0
Malachite green	0.1	5	22	354	24[c]	..	...
Malachite green	0.1	10	31	368	38[c]	..	...
Malachite green	1.0	10	35	406	76	..	...
Quinacrine 2HCl	0.1	5	20	353	23[c]	..	...
Quinacrine 2HCl	0.1	10	20	367	37[c]	..	...

[a] Animals which lived for more than 1020 seconds were considered to have survived and are not included in the mean survival time. [b] Intravenous administration. [c] Increase insignificant. (Student t test, $p > 0.05$.)

employed in this study. The animals were caged in groups of approximately 50 and had access to laboratory chow (Purina) and water *ad libitum* until the beginning of treatment.

The following solutions were prepared with distilled water: *d*-propoxyphene HCl, 2.0%; methylene blue, 0.1 and 1.0%; malachite green oxalate, 0.1 and 1.0%, and quinacrine dihydrochloride, 0.1%.

Ampuls of nalorphine HCl, 5 mg./ml., and levallorphan tartrate, 1 mg./ml., were obtained from their respective manufacturers.

A precision timer was employed to measure survival time. Although this instrument is accurate to one-tenth of a second, the time was measured only to the nearest second.

The intraperitoneal LD_{50} of *d*-propoxyphene, determined by the method of Litchfield and Wilcoxon (12), was 118 (106 to 131) mg./Kg. Based upon the data obtained, a dose of 200 mg./Kg. was chosen as the lethal amount to be used in further experimentation because theoretically it should produce death in 99.5% of the mice.

Each test animal was weighed to the nearest gram, and the proposed antagonist was administered in the upper left abdomen. After a predetermined time had elapsed, the animal was injected intraperitoneally with 200 mg./Kg. of *d*-propoxyphene. The mouse was returned to its cage and kept under observation. The survival time was measured in seconds and extended from the moment of the *d*-propoxyphene injection to the cessation of spontaneous respiration. Any animal that lived for more than 1020 seconds (17 minutes) was considered to have survived. All survivors were kept under observation for 24 hours.

To determine the time of death precisely, the number of animals under observation at one time never exceeded more than six. One mouse in each group of six served as a control and received only the injection of *d*-propoxyphene.

To evaluate their prophylactic capacities against a lethal dose of *d*-propoxyphene, the following agents were administered subcutaneously in the amounts and time intervals indicated in Table I: nalorphine HCl, levallorphan tartrate, methylene blue, malachite green oxalate, and quinacrine dihydrochloride.

The only departure from the subcutaneous route was the intravenous administration of 1.0 mg. of methylene blue 30 minutes prior to the *d*-propoxyphene.

In an effort to obtain more effective antagonism, methylene blue (0.1 mg.) was given immediately after nalorphine HCl (0.1 mg.) and 5 minutes prior to *d*-propoxyphene. In another group, the same amount of methylene blue in conjunction with levallorphan tartrate (0.25 mg.) was administered 5 minutes prior to *d*-propoxyphene.

The data obtained from a total of 475 mice subjected to 200 mg./Kg. of *d*-propoxyphene after treatment with the agents previously mentioned are reported in Table I.

The significance of the differences between the mean survival time of the treated group and the mean survival time of the control group was estimated by the t test, and the significance of the per cent of survival in the treated group with respect to the control group was estimated by the χ^2 test (13). Probability values greater than 0.05 were considered insignificant.

RESULTS AND DISCUSSION

Throughout the investigation, data from a total of 80 mice were compiled to yield control values. No animal survived the intraperitoneal administration of 200 mg./Kg. of *d*-propoxyphene. The sequence of symptoms after injection was: a rapid onset of increased motor activity, ataxia, a Straub tail reaction, followed by prostration and general depression, with clonic convulsions occurring periodically. These convulsant episodes occurred spontaneously but also could be triggered by tactile stimuli, presumably due to hyperreflexia. Spontaneous respiration ceased within a mean time of 330 seconds.

The subcutaneous administration of nalorphine HCl (0.1 mg.) 5 minutes prior to *d*-propoxy-phene protected 18.4% of the mice and extented the mean survival time of the remainder to 510 seconds. These results show a definite antagonism of *d*-propoxyphene by nalorphine HCl in mice and are in greement with reports of similar activity in rats (1, 11).

The nonspecific antagonism by nalorphine HCl against other central nervous system depressants is reported to require doses many times greater than that of the depressant agent. However, with the opiates and allied drugs, the amount of nalorphine HCl needed for effective antagonism is equal to or many times less than the amount of analgesic (14). The quantity of nalorphine HCl used in this study was 4–5 mg./Kg. to protect against 200 mg./Kg. of *d*-propoxyphene or a maximum ratio of 1 part antagonist to 40 parts of antagonist. With respect to dosage, the antagonism exerted by nalorphine HCl against *d*-propoxyphene appeared to be specific in nature and of the same quality as against other potent analgesics.

The administration of levallorphan tartrate was shown also to be effective in antagonizing *d*-propoxyphene toxicity. Although the dose used was two and one-half times greater than that of nalorphine HCl, the results were quite similar quantitatively. It would appear that levallorphan tartrate and nalorphine HCl were effective by the same mechanism of antagonism.

This investigation revealed that methylene blue can protect between 5 and 8% of the mice in each group and prolong significantly the survival time of the remaining animals in three of the five experimental groups.

The protection by methylene blue may be due to two possible types of action. The dye may act throughout the organism to combat the effects of anoxia and shock, or it could exert a specific antagonism against *d*-propoxyphene.

After injection into common laboratory animals, methylene blue has been shown to produce a transitory increase in blood pressure and respiratory rate (15).

Although small doses are reported to produce parasympathetic stimulation, either by a direct action or by the inhibition of acetylcholinesterase, moderate doses of methylene blue exert a definite parasympatholytic action. In rabbits, after the heart rate was drastically reduced by anoxia, the intravenous administration of methylene blue quickly produced an increase in heart rate. The supposition that this was due to a parasympatholytic action was substantiated by the observations that this agent was ineffective after bilateral vagotomy and yet was effective after sympathetic blockade by ergotamine tartrate with the vagi intact and qualitatively was similar to atropine in accelerating the heart (15). Furthermore, after depression with acetylcholine, the perfused frog heart was stimulated by the topical application of methylene blue solution.

Methylene blue was also shown to act directly upon the blood vessels to produce peripheral vasoconstriction which, along with increased heart rate, raised the blood pressure. This direct effect was rather transient but could be reproduced with another administration of the drug (15). An increase in the pressor effect of epinephrine (16) and norepinephrine (17) has been demonstrated in spinal cats after the administration of methylene blue and is postulated to be due to the inhibition of amineoxidase in the liver.

Methylene blue has been shown to produce a transitory rise in the respiratory rate of rabbits, even in severe anoxia, as long as respiration has not ceased completely. In high doses, it acted as a respiratory depressant (15).

It is conceivable that methylene blue acts to maintain respiration and blood pressure in mice subjected to extremely high doses of *d*-propoxyphene. In an attempt to alleviate hemorrhagic shock in rats, Strawitz *et al.* (18) found that the injection of methylene blue significantly reduced mortality.

The possibility that methylene blue may antagonize the central depression of *d*-propoxyphene by a mechanism similar to that of nalorphine HCl or levallorphan tartrate has not been investigated sufficiently at this time to warrant a firm approval or disapproval.

When methylene blue (0.1 mg.) and nalorphine HCl (0.1 mg.) were administered in combination 5 minutes prior to *d*-propoxyphene, the protective effect was greater than the sum of the effects of each agent alone.

Chapman and Walaszek (11) reported nalorphine HCl to be relatively ineffective against the convulsions produced in rats by *d*-propoxyphene. In mice, the convulsions induced by *d*-propoxyphene were diminished slightly by nalorphine HCl; but in most cases, the animals still suffered severe clonic seizures. Methylene blue, or the combination of methylene blue and nalorphine HCl, also appeared to be relatively ineffective in preventing these convulsions.

When levallorphan tartrate (0.25 mg.) and methylene blue (0.1 mg.) were given in combination 5 minutes prior to *d*-propoxyphene, the rate of survival was only 5%, and the increase in survival time was 180 seconds. These results indicated that the administration of methylene blue with levallorphan tartrate did not produce an increase in protection and that the efficiency of levallorphan tartrate was reduced significantly. The reason or reasons why methylene blue apparently hindered the protective effect of levallorphan tartrate while it potentiated the activity of nalorphine HCl cannot be given. This aspect of the problem definitely deserves further investigation.

Malachite green oxalate, a triphenylmethane dye with weak antiseptic properties, produced little protection against 200 mg./Kg. of *d*-propoxyphene. A total of 88 animals were pretreated with this compound, but none survived. However, the mean survival time was extended significantly in the animals receiving 1.0 mg. 10 minutes prior to *d*-propoxyphene.

Quinacrine dihydrochloride, a yellow acridine dye, is used clinically as an antimalarial agent. It also has been shown to inhibit the spastic response produced by acetylcholine, histamine, and barium chloride on the isolated guinea pig ileum and to antagonize cardiac fibrillation in dogs (19). This investigation has shown that pretreatment with quinacrine dihydrochloride (0.1 mg.) 5 or 10 minutes prior to *d*-propoxyphene exerted an insignificant protective effect.

REFERENCES

(1) Robbins, E. B., THIS JOURNAL, **44**, 497(1955).
(2) Pohland, A., and Sullivan, H. J., *J. Am. Chem. Soc.*, **77**, 3400(1955).
(3) Marrs, J., Glass, W., and Silvani, J., *Am. J. Pharm.*, **131**, 271(1959).
(4) Gruber, C. M., *et al.*, *J. Pharmacol. Exptl. Therap.*, **118**, 280(1956).
(5) Van Bergen, W., and North, W., *J. Am. Med. Assoc.*, **172**, 1372(1960).
(6) Cann, H. M., and Verhulst, H. L., *U. S. Bull. Natl. Clearinghouse Poison Control*, Washington, D. C., March 1960.
(7) Hyatt, H. W., *New Engl. J. Med.*, **267**, 710(1962).
(8) Frasier, S. D., Crudo, F. S., and Johnson, D. H., *J. Pediat.*, **63**, 158(1963).
(9) Cann, H. M., and Verhulst, H. L., *op. cit.*, July 1960.
(10) Cann, H. M., and Verhulst, H. L., *A. M. A. J. Diseases Children*, **99**, 380(1960).
(11) Chapman, J. E., and Walaszek, E. J., *Toxicol. Appl. Pharmacol.*, **4**, 752(1962).
(12) Litchfield, J. T., and Wilcoxon, F. J., *J. Pharmacol. Exptl. Therap.*, **96**, 99(1949).
(13) Simpson, G. G., Roe, A., and Lewontin, R. C., "Quantitative Zoology," Harcourt, Brace, and Co., New York, N. Y., 1960, pp. 176, 186.
(14) Costa, P. J., and Bonnycastle, D. D., *J. Pharmacol. Exptl. Therap.*, **113**, 310(1955).
(15) Jalavisto, E., Kantero, I., and Leikola, Y., *Acta Physiol. Scand.*, **23**, 212(1951).
(16) Philpot, F., and Cantoni, G., *J. Pharmacol. Exptl. Therap.*, **71**, 95(1941).
(17) West, G., *J. Pharm. Pharmacol.*, **3**, 656(1951).
(18) Strawitz, J., Temple, R., and Heft, H., *Surg. Forum*, **9**, 54(1958).
(19) Gertler, M., and Karp, M., *Proc. Soc. Exptl. Biol. Med.*, **64**, 213(1947).

Distribution of Glucose-1-C-14 in Gold Thioglucose Obese and Normal Nonobese Mice

By D. L. ARNEY* and H. A. SWARTZ

Normal nonobese and gold thioglucose obese mice show similar patterns of residual tissue activity following glucose-1-C-14 administration in controls, glucose pretreated, and 48-hour starvation. Glucose pretreatment lowered liver and muscle levels and elevated blood and kidney levels; starvation elevated liver and muscle but lowered kidney and blood levels. The obese mice showed greater liver activities associated with increased glycogen formation. Hypothalamic levels were higher than the cerebrum and hind brain in all instances with the obese mice but only after starvation in the nonobese mice. Selected anorectic agents had little effect on general tissue activities but caused significant increases in hypothalamic levels in normal nonobese mice.

THE REGULATION of food intake by centers in the hypothalamus has been established in several species (1), including dogs (2, 3), rats (4–6), monkeys (7, 8), cats (9), and mice (10). These studies involved the production of surgical lesions into specific hypothalamic nuclei. In all instances where the ventro medial nuclei were destroyed completely or partially, hyperphagia was observed, while destruction of the lateral hypothalamic nuclei resulted in a temporary aphagia. Administration of LD_{50} doses of gold thioglucose to mice was reported to result in hyperphagia and subsequent obesity (11, 12). Histological examination within 3 days of administration revealed hypothalamic lesions associated predominately with the ventro medial nuceli (13) but also in other neighboring nuclei. Examination after a period of 3 months revealed that permanent damage was restricted to the ventro medial nuclei. With rats, the destruction of the ventro medial cells was fatal in all instances (14). Administration of similar gold compounds, such as gold thiomalate, gold thiosorbitol, gold thioglycerol, gold thiocaproate, and gold thioglycoanline, did not produce hypothalamic lesions in either rats or mice or result in hyperphagia.

The integrity of the ventro medial nuclei appeared essential for food intake regulation; as only the gold thioglucose molecule was capable of producing lesions, glucose was considered to be involved in the physiological action of these cells. It was postulated that the ventro medial nuclei had an affinity for available glucose and acted as a food intake satiety center in response to blood glucose levels (15–17). This postulation resulted in the glucostatic theory of food intake regulation. Sulfur-35 and gold-198 labeled gold thioglucose were reported to concentrate in the hypothalamus, as observed from autoradiogram and tissue activity studies (18).

The lateral hypothalamic nuclei have been characterized as appetite or feeding centers (19–21), and stimulation of these cells results in hyperphagia and their destruction in aphagia. The ventro medial nuclei, in response to blood glucose, were shown to inhibit the lateral feeding center (22, 23). Hence, the destruction of the ventro medial nuclei prevents the indirect inhibiting action of glucose on the feeding center, and hyperphagia occurs.

Gold thioglucose obese mice offered an excel-

Received August 26, 1964, from the College of Pharmacy, Butler University, Indianapolis, Ind.
Accepted for publication September 29, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
* Present address: Long Hospital, Indiana University Medical Center, Indianapolis.