lem would be most pronounced with Products A, Pp, Pr, and V. The slow absorption coupled with the inability to control the actual amount of drug available to a given patient raises serious questions as to the usefulness of the presently available aspirin suppository dosage forms for aspirin or salicylate therapy.

REFERENCES

(1) M. M. Nowak, B. Grundhofer, and M. Gibaldi, *Pediatrics*, 54, 23(1974).

(2) B. B. Brodie, S. Udenfriend, and A. F. Coburn, *J. Pharmacol. Exp. Ther.*, 80, 114(1944).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 30, 1974, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication November 29, 1974.

Supported in part by Grant GM20852 from the National Institute of General Medical Sciences, National Institutes of Health.

[x] To whom inquiries should be directed.

Central *versus* Peripheral Anticholinergic Activity as Assessed by Two *In Vivo* Procedures in Mice

JEFFREY B. MALICK [x] and ALLEN BARNETT

Abstract □ The activity of tertiary and quaternary anticholinergic drugs was compared in two different test procedures designed to measure cholinolytic activity in mice. The four drugs utilized were atropine sulfate, atropine methylnitrate, scopolamine hydrobromide, and scopolamine methylnitrate. The results led to the conclusion that one of these test procedures, the induction of mydriasis (increase in pupil size), primarily measures peripheral anticholinergic activity whereas the other procedure, inhibition of physostigmine lethality, primarily measures anticholinergic activity in the CNS. These two test procedures can be utilized to characterize the nature of the cholinolytic properties of prospective therapeutic drug candidates.

Keyphrases □ Anticholinergic activity—determination of central *versus* peripheral cholinolytic activity, tertiary and quaternary derivatives of atropine and scopolamine, mice □ Mydriasis induction as measure of peripheral anticholinergic activity—tertiary and quaternary derivatives of atropine and scopolamine, compared to physostigmine-induced lethality inhibition (central activity), mice □ Physostigmine-induced lethality inhibition as measure of central anticholinergic activity—tertiary and quaternary derivatives of atropine and scopolamine, compared to mydriasis induction (peripheral activity), mice □ Atropine and scopolamine, tertiary and quaternary derivatives—central *versus* peripheral cholinolytic activity, mice □ Scopolamine and atropine, tertiary and quaternary derivatives—central *versus* peripheral cholinolytic activity, mice

The measurement of anticholinergic activity is important in the evaluation of drug candidates for humans. Peripheral anticholinergic liability is considered to be an undesirable side effect of many therapeutic agents, particularly with regard to antidepressants (1) and antihistamines (2). Central [*i.e.*, within the central nervous system (CNS)] anticholinergic activity may not be as troublesome (except at toxic doses) and, in fact, may even be desirable under certain circumstances. Central anticholinergic drugs (*e.g.*, scopolamine) produce sedation in humans, and amitriptyline, a potent central anticholinergic and sedative drug, may be the antidepressant of choice in cases of agitated depression, a condition in which sedative effects have utility (3).

In the present studies, two test procedures in mice were evaluated for their ability to detect central *versus* peripheral anticholinergic activity. To evaluate these procedures, tertiary and quaternary forms of atropine and scopolamine were utilized. The tertiary forms readily cross the blood–brain barrier whereas the quaternary forms are retarded by the blood–brain barrier and, consequently, are concentrated to a much lesser extent in the CNS than they are in the periphery following parenteral administration. Thus, by comparing the relative potency of these agents in the two procedures, the induction of mydriasis and the antagonism of physostigmine-induced lethality, the extent that each can predict central or peripheral anticholinergic activity was determined.

EXPERIMENTAL

Male CF No. 1-S mice, 18–22 g, were used. All drug doses were calculated in terms of milligrams per kilogram of free base, and all drugs were dissolved in distilled water. The volume of injection was 10 ml/kg for both the intraperitoneal and subcutaneous routes of administration. The drugs used were atropine sulfate, atropine methylnitrate, scopolamine hydrobromide, and scopolamine methylnitrate.

Induction of Mydriasis (Increased Pupil Size) in Mice—Mice were administered test drugs and were tested for mydriatic activity 30 min later. These studies were performed under double-blind conditions such that the investigator did not know what drugs or doses were being tested. Normal pupil size was scored as 0 and increases in pupil size were scored as 1, 2, and 3 (slight, moderate, and maximal increases in size, respectively). A score of 2 was approximately 50% of maximal pupil size, which was a modification of the scoring system of Janssen and Niemegeers (4).

Any mouse exhibiting a pupil size of 2 or greater was considered to be significantly affected. Mean pupil sizes for each group were also calculated. The dose intervals for each test drug were kept constant (0.5 $\log_{10}$ units). The MED_{50} (minimal effective dose) for producing a significant increase in mydriasis in 50% or more of the mice tested was determined for each test drug. The ED_{50}'s could not be calculated since there were very small intervals between doses at which no animals exhibited significant effects and doses producing effects in 100% of the mice.

Inhibition of Physostigmine-Induced Lethality in Mice—

Table I—Induction of Mydriasis (Increase in Pupil Size) by Tertiary and Quaternary Anticholinergic Drugs in Mice

Treatment	Dose, mg/kg ip[a]	Mean Pupil Size[b]	Number of Mice Exhibiting Significant Mydriasis[c] / Number of Mice Tested	MED_{50}, mg/kg ip, for Inducing Mydriasis[d]
Saline control	1	0.0	0/30	—
Atropine sulfate	0.1	0.7	0/6	0.3
	0.3	2.3	5/6	
	1.0	2.8	6/6	
	3.0	3.0	6/6	
Atropine methylnitrate	0.01	0.0	0/6	0.1
	0.03	0.3	0/6	
	0.1	2.8	6/6	
	0.3	3.0	6/6	
Scopolamine hydrobromide	0.01	1.0	0/6	0.1
	0.03	1.3	2/6	
	0.1	1.7	5/6	
	0.3	2.7	6/6	
	1.0	2.5	6/6	
Scopolamine methylnitrate	0.003	0.2	0/6	0.03
	0.01	0.5	0/6	
	0.03	2.3	6/6	
	0.1	3.0	6/6	

[a] Drug administered 30 min prior to testing. [b] Pupil size scored as follows: 0 = normal size, 1 = slight increase, 2 = moderate increase, and 3 = marked increase. [c] Number of mice exhibiting a significant increase in pupil size (score of 2 or greater). [d] Minimal effective dose for producing significant mydriasis in 50% or more of the animals tested.

The method used was a modification of the technique reported by Collier *et al.* (5). Physostigmine salicylate (1.0 mg/kg sc) produced 100% lethality when administered to mice grouped 10 per cage [12.7 × 28 × 12.7 cm (5 × 11 × 5 in.)] 20 min after drug administration. Test agents were administered intraperitoneally 30 min prior to physostigmine. Inhibition of lethality was then calculated as percent of control. The ED_{50}, the dose that prevented lethality in 50% of the mice, was calculated for each drug by the Maximum Likelihood Probit Analysis Method (6).

RESULTS AND DISCUSSION

The induction of mydriasis by the four anticholinergic drugs is summarized in Table I. All four drugs produced dose-related increases in pupil size, as indicated by mean pupil size. The MED_{50}'s for induction of mydriasis by atropine sulfate, atropine methylnitrate, scopolamine hydrobromide, and scopolamine methylnitrate were 0.3, 0.1, 0.1, and 0.03 mg/kg ip, respectively. The quaternary forms of the anticholinergic drugs were more potent as mydriatics than their respective tertiary forms; both atropine methylnitrate and scopolamine methylnitrate were approximately three times as potent as their respective tertiary forms.

Table II summarizes the results of studies to determine the relative potency of the four anticholinergic drugs as inhibitors of physostigmine-induced lethality in mice. The ED_{50}'s for atropine sulfate, atropine methylnitrate, scopolamine hydrobromide, and scopolamine methylnitrate were 1.6, 12.8, 0.35, and 6.1 mg/kg ip, respectively. Although all four drugs inhibited the lethality produced by physostigmine, a cholinesterase inhibitor, the tertiary forms, in contrast to the results obtained in the mydriasis studies, were considerably more potent than their respective quaternary forms. Atropine sulfate was eight times more potent than atropine methylnitrate, and scopolamine hydrobromide was 18 times more potent than scopolamine methylnitrate in this procedure. This result was believed to be due to the blood–brain barrier impeding the passage of the quaternary derivatives into the CNS.

Table III summarizes the potency ratio comparisons of the various drugs in the two anticholinergic test procedures. As mentioned, there was a reversal of the potency relationships when appropriate tertiary and quaternary derivatives were compared such that the quaternary forms were more potent mydriatic agents whereas the tertiary forms were more potent inhibitors of physostigmine lethality. When scopolamine hydrobromide was compared to atropine sulfate, the former was approximately three to four times more potent than the latter in both procedures. The same relationship between the quaternary derivatives held true; *i.e.*, scopolamine methylnitrate was approximately two to three times more potent than atropine methylnitrate as an anticholinergic in both procedures (Table III).

From these results, it was concluded that the induction of my-

Table II—Antagonism of Physostigmine-Induced Lethality in Mice

Treatment[a]	Number of Mice Used	ED_{50}, mg/kg ip, for Antagonism of Physostigmine Lethality (95% Confidence Limits)[b]
Atropine sulfate	90	1.6 (1.2–2.3)
Atropine methylnitrate	150	12.8 (6.3–54.9)
Scopolamine hydrobromide	140	0.35 (0.19–0.40)
Scopolamine methylnitrate	100	6.1 (4.1–8.4)

[a] Drugs administered 30 min prior to physostigmine (1.0 mg/kg sc). [b] The ED_{50} and 95% confidence limits were calculated by Probit Maximum Likelihood Analysis (6).

Table III—Potency Ratio Comparisons of the Activities of Anticholinergic Drugs in Two Procedures in Mice

Comparison	Induction of Mydriasis Potency Ratio[a]	Antagonism of Physostigmine Lethality Potency Ratio (95% Confidence Limits)[b]
Atropine sulfate *versus* atropine methylnitrate	0.33	8.1 (5.0–14.7)
Scopolamine hydrobromide *versus* scopolamine methylnitrate	0.30	18.1 (12.3–27.7)
Scopolamine hydrobromide *versus* atropine sulfate	3.00	4.7 (3.2–7.2)
Scopolamine methylnitrate *versus* atropine methylnitrate	3.33	2.1 (1.3–3.5)

[a] Calculated from MED_{50}'s from Table I. [b] The potency ratio and 95% confidence limits were calculated by Maximum Likelihood Potency Probit Analysis (6) from data in Table II.

driasis was primarily a peripheral anticholinergic response since both the tertiary and quaternary derivatives of atropine and scopolamine were potent inducers of mydriasis. It also was concluded that inhibition of physostigmine-induced lethality was primarily a measure of central anticholinergic activity since the quaternary derivatives were much less potent inhibitors of physostigmine-induced lethality than their respective tertiary forms.

A careful evaluation of a drug's relative activity in these two procedures in the same species, the induction of mydriasis and the inhibition of physostigmine lethality, should predict its relative activity as a central and a peripheral anticholinergic in humans.

REFERENCES

(1) G. L. Klerman and J. O. Cole, *Pharmacol. Rev.*, **17**, 101(1965).

(2) F. E. Roth and I. I. A. Tabachnick, in "Drill's Pharmacology in Medicine," J. R. DiPalma, Ed., McGraw-Hill, New York, N.Y., 1971, p. 995.

(3) G. E. Vaillant, *Amer. J. Psychiat.*, **125**, 1600(1969).

(4) P. A. J. Janssen and C. J. E. Niemegeers, *Psychopharmacologia*, **11**, 231(1967).

(5) H. O. J. Collier, L. C. Dineen, C. A. Johnson, and C. Schneider, *Brit. J. Pharmacol.*, **32**, 295(1968).

(6) D. J. Finney, "Statistical Methods in Biological Assay," Hafter, New York, N.Y., 1964.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 16, 1974, from the *Department of Pharmacology, Schering Corporation, Bloomfield, NJ 07003*

Accepted for publication December 4, 1974.

The authors are grateful to Mr. Rene Roblejo for technical assistance.

[x] To whom inquiries should be directed. Present address: Department of Pharmacology, ICI United States, Inc., Wilmington, DE 19899

Tissue Distribution of *N*-^{14}C-Azure C (Methylthionine) in the Rat

R. ENG, S. LINDBERG, K. WHITE, and L. A. SPITZNAGLE [x]

Abstract □ The distribution of radioactivity at 5-, 10-, and 15-min intervals following the intravenous administration of *N*-^{14}C-azure C was determined. The concentrations of radioactivity observed indicated that radioactive derivatives of azure C would not be useful pancreas or parathyroid scanning agents.

Keyphrases □ Azure C, radiolabeled—tissue distribution, considerations related to use as pancreas or parathyroid scanning agent □ Methylthionine, radiolabeled—tissue distribution, considerations related to use as pancreas or parathyroid scanning agent □ Scanning agents—tissue distribution of radiolabeled azure C

The report (1) that the intravenous administration of the phenothiazine dye tolonium chloride (toluidine blue O) caused a blue coloration of the pancreas and parathyroid glands but not of surrounding organs generated much interest and has been verified in experimental animals (2–4) and humans (5–7). Toluidine blue O has been used for the identification of the parathyroid gland at surgery (5–7) and as an aid in the diagnosis of small oral cancers (8), and it has been suggested as a potential parathyroid and pancreas scanning agent if labeled with a suitable radionuclide (2–4, 6, 7). Recent studies with an iodinated analog of toluidine blue O labeled with ^{131}I showed it to be useful for imaging parathyroid adenomas (9).

DISCUSSION

Kang and DiGiulio (3) found the highest concentrations of toluidine blue O in the parathyroids, heart, pancreas, kidneys, stomach, lungs, thyroid, muscles, liver, and blood, in that order. They extracted the excised tissues with ethanol and measured the dye concentrations colorimetrically. Mortenson and McRae (2) reported similar results using a similar assay. In both studies, rather low concentrations were observed in the organs surrounding the pancreas, suggesting the development of a pancreas scanning agent provided a suitable labeled derivative could be prepared.

Larose *et al.* (10) studied the distribution in rats of an iodinated derivative of toluidine blue O labeled with ^{125}I. Using an assay method that allowed the measurement of tissue radioactivity without extraction, they found the concentrations of radioactivity in the pancreas, liver, and kidneys to be similar for up to 60 min after intravenous administration. Likewise the parathyroid was observed to have concentrations of radioactivity similar to those found in the thyroid gland. They concluded that iodinated toluidine blue O was an unsatisfactory scanning agent for the pancreas and parathyroid glands.

Archer *et al.* (11) also studied the tissue distribution of a ^{125}I-labeled derivative of toluidine blue O. However, they extracted the tissues with ethanol and measured the radioactivity of the extracts. Their results differed from those of Larose *et al.* (10) in that the parathyroid extract contained approximately three times the concentration of extractable radioactivity as did the thyroid. The pancreas extract contained only a slightly higher concentration of radioactivity than the liver extract, indicating that iodinated toluidine blue O was an unsatisfactory scanning agent for the pancreas. However, these investigators observed that the percentage of the injected dose taken up by the pancreas was much greater when unlabeled toluidine blue O was given instead of iodinated toluidine blue O.

Preliminary work in this laboratory confirmed the results of Klopper and Moe (1); 30 min after the intravenous administration of toluidine blue O, extracts of the pancreas had 10 times the concentration of dye compared to extracts of the liver when measured colorimetrically (12). In a preliminary investigation of the distribution of ^{35}S-labeled toluidine blue O (12), concentrations of radioactivity in the pancreas, liver, and kidneys were found to be similar. These results seemed to confirm the conclusions of Larose *et al.* (10) and Archer *et al.* (11) that labeled derivatives of toluidine blue O were unsatisfactory pancreas scanning agents.

One possible explanation of the blue coloration of the pancreas consistent with these results is that the dye molecule may be