TABLE II.—NORTRIPTYLINE HYDROCHLORIDE EXCRETION AFTER SINGLE DOSES

Dose, mg.	Collection Interval, hr.	Nortriptyline HCl	"Free" Metabolite	"Bound" Metabolite	Total	% of Dose
25	0–24	Negligible	4.6	4.9	9.5	38.0
30	0–24	1.0	3.2	3.4	7.6	25.3
	24–48	0.8	2.1	2.0	4.9	16.3
	48–72	0.8	0.7	1.7	3.2	10.7
	Totals	2.6	6.0	7.1	15.7	52.3

TABLE III.—NORTRIPTYLINE HYDROCHLORIDE EXCRETION AFTER 3 SUCCESSIVE DAILY 10-mg. DOSES

Day	Dose, mg.	Nortriptyline HCl	Nortriptyline Equivalents, mg.[a] "Free" Metabolite	"Bound" Metabolite	Total
1	10	0.96	0.98	1.91	3.85
2	10	0.95	0.84	2.35	4.14
3	10	0.90	2.36	2.52	5.78
4	None	0.93	2.03	3.22	6.18

[a] Urine was collected during the 24-hr. interval between doses and during the 24-hr. interval following the final dose.

rate was found to occur in the 4–8-hr. interval after dosage. The drug is slowly excreted in the urine and substantial levels were found 2–3 days after dosage. Within the 0–24-hr. interval a lower percentage of the administered dose is recovered in the urine than from those patients on chronic administration of the drug.

The data in Table III show the gradual increase in the amount of drug excreted daily as the dose is maintained over a period of 3 days. These data were obtained on the same subject as was used in the single dose study.

From the data presented, it is evident that the assay method described is capable of permitting a reliable evaluation of nortriptyline hydrochloride ingestion. If one is interested only in whether a patient has taken his medication and is not interested in the total amount of nortryptyline present in the urine, it would be necessary to carry out only *Method A* of the assay procedure. The presence of the drug can also be detected by the TLC system described.

SUMMARY

1. A quantitative U.V. spectrophotometric assay method has been developed for the determination of nortriptyline hydrochloride and its principal metabolites in the urine of man.

2. The drug is slowly excreted in the urine primarily as conjugated and unconjugated 10-hydroxy nortriptyline. Little unchanged nortriptyline is excreted.

3. An average of 58% of the daily dose was recovered in 24-hr. urine samples from patients on a continuing dosage regimen of the drug. An average of 34% of the dose was recovered from the 24-hr. urine of a subject given single doses.

4. A thin-layer chromatographic system is described which permits rapid identification of the presence of the drug and/or metabolites in the urine.

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Adrenolytic Activity of Atropine, (+)-Hyoscyamine, Atroscine, Homatropine, and Related Compounds

By F. P. LUDUENA and MARY-JANE BRANIN

The adrenolytic activity of atropine, (+) and (−) hyoscyamine, (−)-hyoscine, atroscine, and related compounds was determined by their ability to antagonize the lethal effect of epinephrine in rats. (+)-Hyoscyamine was found to be more active than atropine, and atropine was slightly more active than atroscine (racemic hyoscine). The *levo*-isomers, (−)-hyoscyamine and (−)-hyoscine, were inactive. These results indicate that (+)-hyoscyamine and (+)-hyoscine are responsible for the effect of atropine and atroscine, respectively. Homatropine and benztropine, but not tropine itself, were active. Benztropine was approximately one-fiftieth as active as phentolamine. A pair of esters of tropine had weak adrenolytic activity, but their pseudotropine isomers were inactive. Atropine aminoxide (genatropin) and atropine methyl nitrate had no adrenolytic activity.

IT HAS long been known that atropine, in high doses, inhibited or reduced the effect of epinephrine and sympathetic stimulation on various arterial beds (1). Those results included the effect of epinephrine on arterial strips from horses, oxen, and rabbits and perfused arterial

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beds of frogs, dogs, and rabbits. Bussell (1) showed that atropine reduced the effect of epinephrine on the blood pressure of the spinal cat, the dog perfused leg, the cat nictitating membrane, the perfused rabbit ear, and the rabbit uterus. He found that (−)-hyoscyamine was inactive or had very little activity. More recent studies have confirmed these results. Graham (2) demonstrated that atropine and (−)-homatropine blocked the constricting effect of epinephrine on the duck duodenum, while (−)-hyoscyamine and (−)-hyoscine were inactive. He also reported that the relaxation of the hen's caecum induced by epinephrine was not changed by atropine. Ruegg (3) found that atropine reduced the effect of epinephrine and norepinephrine on the rabbit iris dilator *in vitro*.

The general conclusion to be drawn from the literature data is that atropine blocks or reduces the effect of epinephrine and other catecholamines on sympathetic α-receptors and has no effect on β-receptors. In other words, atropine has adrenolytic (α-sympatholytic) activity.

The experiments described below were carried out to compare the adrenolytic activity of atropine with that of related compounds and, since (−)-hyoscyamine had been reported to be inactive, to determine whether the activity of atropine (racemic hyoscyamine) was that of the *dextro*-isomer in the mixture.

EXPERIMENTAL

Method.—The method was based on the ability of adrenolytics to antagonize the lethal effect of epinephrine in rats. The rationale for the selection of the rat as experimental animal, the intravenous route of administration, and the injection of epinephrine with the antagonist in the same solution has been discussed earlier (4). Two slightly different techniques were used. *Method A*, described earlier (4), follows.

Method A.—Male white rats, weighing between 80–110 Gm., were injected intravenously with a 200-mcg./Kg. (2.7–5 × LD_{50}) dose of epinephrine together with one of a series of graded doses of the compound to be tested. Using 0.3-log intervals, the dose of the compound under study was varied, while the concentration of epinephrine was kept constant. To obtain the ED_{50}, the percentage mortality was plotted against dose on log probit paper.

Method B.—*Method B* was employed when the adrenolytic activity of the compound was too weak to be observed by *Method A*. Similarly, a combined injection of epinephrine and the compound to be tested was administered intravenously to male white rats. In this case, however, the dose of the compound was kept constant, while the concentration of epinephrine was varied, at 0.2-log intervals. Again, plotting per cent mortality *versus* dose, the LD_{50} of epinephrine, as affected by the weak adrenolytic, was determined. In comparing this value with values obtained by determining the toxicity of epinephrine alone, slight adrenolytic activity could be detected. In Table II the results obtained with the 2 techniques are shown in different columns. In the case of *Method B*, the control LD_{50} for epinephrine, obtained with animals from the same batch, is included also. Doses of phentolamine, smaller than the ED_{50} obtained by *Method A*, were tested by *Method B*. The results in Table II show that even the smaller of the 2 doses used reduced the intravenous toxicity of epinephrine.

Compounds Used.—Atropine sulfate [(±)-hyoscyamine (Merck)], (+)-hyoscyamine 1-2-oxobornanesulfonate, (−)-hyoscyamine sulfate, homatropine hydrobromide (Merck), benztropine methanesulfonate[1] (3-diphenyl methoxytropane methane sulfonate), apoatropine hydrochloride, and tropine hydrochloride.

Compound I (tropyl diphenylacetate hydrochloride), compound II (pseudo-tropyl diphenylacetate hydrochloride), compound III (tropyl-2-cyclopentyl - 4 - methylpentanoate hydrochloride), compound IV (pseudo - tropyl -2 - cyclopentyl - 4-methylpentanoate hydrochloride), atropine aminoxide chlorhydrate (American Roland Co.), atropine methyl nitrate,[2] and (−)-hyoscine-hydrobromide (scopolamine hydrobromide) (Merck.).

(±)-Hyoscine (atroscine) prepared by racemization of (−)-hyoscine by Dr. Benjamin F. Tullar, diphenhydramine[3] (Parke, Davis and Co.), compound V (3-phenyltropane hydrochloride), phentolamine HCl,[4] papaverine hydrochloride (Merck), and pronethalol hydrochloride[5] (nethalide HCl).

RESULTS

Examination of the results summarized in Table I shows that some of the compounds tested had adrenolytic activity.

Of the 2 optical isomers of atropine only (+)-hyoscyamine antagonized the lethal effect of epinephrine; (−)-hyoscyamine in a dose of 40 mg./Kg. failed to protect the rats injected with a 200-mcg./Kg. dose of epinephrine.

The activity of (+)-hyoscyamine was low; phentolamine tested in the same manner was approximately 400 times more active.

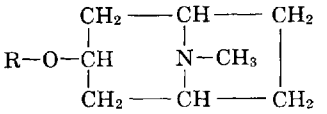
The results showed clearly that there is no correlation between cholinolytic and adrenolytic activities. The cholinolytic action of (−)-hyoscyamine was found by Long *et al.* to be 110–250 times higher than that of the (+) isomer. This (−)/(+)-activity ratio is considerably higher than those reported by others (6–8). [Lower ratios may indicate (−)-isomer contamination of the (+)-hyoscyamine sample.]

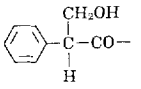
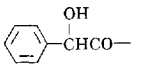
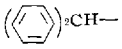
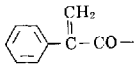
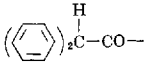
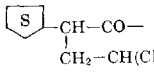
Atropine aminoxide (genatropine) and atropine methyl nitrate had no adrenolytic activity.

Scopolamine [(−)-hyoscine] was inactive at the highest dose tested (40 mg./Kg.). Racemic hyoscine (atroscine) was slightly less active than atropine; this indicates that (+)-hyoscine is the

[1] Marketed as Cogentin by Merck Sharp & Dohme.
[2] Marketed as Eumydrin by S. B. Penick Co.
[3] Marketed as Benadryl by Parke, Davis and Co.
[4] Marketed as Regitine HCl by Ciba Pharmaceutical Co.
[5] Marketed as Aldetlin HCl by Imperial Chemical Industries Ltd.

TABLE I.—ADRENOLYTIC ACTIVITY OF ATROPINE AND RELATED COMPOUNDS



Compd.	Salt	Structure R	Tropine (T) Pseudo-Tropine (P)	Adrenolytic Activity A ED_{50}, mg./Kg.[a]	Adrenolytic Activity B Increase in Epinephrine LD_{50} Epinephrine Alone, mcg./Kg. (Base)	Epinephrine + x mg. Drug/Kg. (mcg./Kg. Base)	Drug/Kg. (Base)
Atropine (± hyoscyamine)	Sulfate, H_2O				42.7 ± 5	232 ± 30	33.4
(+)-Hyoscyamine	1-2-Oxobornane-sulfonate		T	8.5 ± 1.5			
(−)-Hyoscyamine	Sulfate				Inactive		34.2
Homatropine	Hydrobromide		T		37.5 ± 4.2	148 ± 11	30.9
Benztropine	Methanesulfonate		T	1.14 ± 0.21			
Apoatropine	Hydrochloride		T		Inactive		10
Tropine	Hydrochloride				Inactive		100
I	Hydrochloride		T		42.7 ± 4.5	180 ± 13.5	10
II	Hydrochloride		P		Inactive		10
III	Hydrochloride		T		37.5 ± 4.2	180 ± 24	10
IV	Hydrochloride		P		Inactive		10
Atropine aminoxide	Hydrochloride				Inactive		40

[a] In terms of the bases.

TABLE II.—ADRENOLYTIC ACTIVITY OF ATROPINE AND RELATED COMPOUNDS

Compd.	Salt	Structure	Adrenolytic Activity: A — ED_{50}, mg./Kg. (In Terms of the Bases)	Adrenolytic Activity: B — Epinephrine Alone, mcg./Kg. (Base)	Adrenolytic Activity: B — Increase in Epinephrine LD_{50} — Epinephrine + x mg. Drug/Kg. (mcg./Kg. Base)	Adrenolytic Activity: B — Increase in Epinephrine LD_{50} — x
Atropine methyl	Nitrate				Inactive	10
(−)-Hyoscine (scopolamine)	Hydrobromide · $3H_2O$				Inactive	40
(±)-Hyoscine (atroscine)	Hydrochloride			42.7 ± 4.5	134 ± 17.5	40
Diphenhydramine	Hydrochloride				Inactive	10
V	Hydrochloride				Inactive	10
Phentolamine	Hydrochloride		0.022 ± 0.0038			
Phentolamine	Hydrochloride			37.5 ± 5	87 ± 16	0.0057
Phentolamine	Hydrochloride			37.5 ± 5	55 ± 4.6	0.0027
Papaverine	Hydrochloride				Inactive	4
Pronethalol	Hydrochloride				Inactive	4

active isomer and that substitution of scopine for the tropine ring in atropine does not abolish adrenolytic activity.

Pronethalol and papaverine, at the doses tested, did not protect rats from the lethal effect of epinephrine.

The most active of the compounds tested was benztropine, its activity being approximately one-fiftieth of that of phentolamine. Homatropine and 2 other tropanol esters (I and III) were slightly less active than atropine. The pseudotropanol analogs (II and IV) of the last 2 compounds had no activity.

Substitution of a dimethylamino ethanol group in compound I for the tropanol moiety abolished adrenolytic activity. (I *versus* diphenhydramine.)

Most of the compounds tested had cholinolytic activity. Since paralysis of the vagi and cholinergic vasodilators by cholinolytics may increase the magnitude and the duration of epinephrine hypertensive effect, the following experiment was performed. Graded doses of epinephrine were injected together with a dose of 1 mg./Kg. of (−)-hyoscyamine. As expected, the toxicity of epinephrine was slightly increased. [LD_{50} of epinephrine alone was 37.5 ± 4 mcg./Kg.; when 1 mg./Kg. of (−)-hyoscyamine was added to each of the graded doses of epinephrine the LD_{50} was 27 ± 2.7 mcg./Kg.] These results suggest that the adrenolytic effect of the active compounds tested had to be exerted against an epinephrine toxicity somewhat greater than that obtained on control rats.

DISCUSSION

The experiments reported above have confirmed, by a different method, reports in the literature which showed that atropine (1) and homatropine (2) had adrenolytic activity and that (−)-hyoscyamine was inactive (1, 2). Although (+)-hyoscyamine had not been tested before, the fact that (−)-hyoscyamine was inactive strongly suggested that the known weak adrenolytic activity of atropine was due to (+)-hyoscyamine. Our experiments have demonstrated that (+)-hyoscyamine is, indeed, an adrenolytic of a potency greater than that of atropine.

Since (−)-hyoscine (scopolamine) is inactive the activity of racemic hyoscine (atroscine) must be due, as in the case of atropine, to the action of the *dextro*-isomer.

In the series of compounds studied, with the exception of atroscine, the adrenolytic activity is associated with the presence of the tropanol moiety in the molecule, although tropine itself is inactive. Substitution of scopine for the tropine ring in atropine did not abolish activity but only reduced it. The presence of a tertiary amine group appears to be important—both atropine aminoxide and atropine methyl nitrate were inactive.

The activity of (+)-hyoscyamine was approximately $^1/_{100}$ of that of phentolamine. Assuming that the activity of atropine is 50% of that of (+)-hyoscyamine, the phentolamine/atropine activity ratio of 800 differs from that reported by Fleckenstein (9), who found a molar activity ratio of 375 in experiments on the perfused rabbit ear. He also found that diphenhydramine, inactive in our test, was as active as atropine. Since in Fleckenstein's experiments the adrenolytic was perfused, the con-

centration of diphenhydramine at the biophase, when distribution equilibrium had been reached, may have been much higher than that of atropine. In our experiments higher doses of diphenhydramine could not be administered because of toxicity.

The steric configuration of the tropyl moiety in atropine and atroscine plays an important role, since in both cases only the *dextro*-isomers are active. Cushny (10) reported that atropine was 200 times more active as a cholinolytic than its desoxy analog. This ratio is within the range of the *levo/dextro* hyoscyamine activity ratios obtained by Long *et al.* (5), suggesting, in accordance with Easson and Stedman's (11) theory, that the side chain OH group contributes to the attachment to the parasympathetic receptor. With the information available we can only theorize on the influence of the OH group in the tropyl moiety on adrenolytic activity. Only one of the enantiomorphs may have a configuration that provides an extra bond (hydrogen) for attachment to α-sympathetic receptors. Or possibly, adrenolytic activity may occur in atropine and racemic hyoscine only when the OH group is "pointing away" from the receptor, as appears to be the case with the adrenolytic activity of (+)-isoproterenol (12). In this case the OH group is not essential since the β-desoxy analog is equally active. The desoxy analogs of atropine and hyoscine could not be tested because they were unavailable. However, a closely related compound of atropine, apoatropine, was found inactive at 10 mg./Kg. (The toxicity of the compound prevented the use of higher doses.)

In examining the method we employed, the question may be raised of whether the antagonism of the lethal effect of epinephrine is a measure of adrenolytic activity (blockade of α-sympathetic receptors), or could protection against epinephrine toxicity be obtained with other types of drugs? To investigate this problem, 2 drugs which were known to antagonize some of the effects of epinephrine without acting on α-receptors—a smooth muscle relaxant, papaverine, and a β-receptor blocker, pronethanol—were tested.

As discussed in a previous paper (4), the data in the literature indicate that the fatal pulmonary edema which follows the intravenous injection of high doses of epinephrine is due to a pronounced rise of arterial and pulmonary venous pressure. Theoretically, antagonists of the effect of epinephrine on the blood vessels or the heart could act as epinephrine antidotes. However, pronethalol, a β-receptor blocker, in doses of 1, 2, or 4 mg./Kg., had no antidotal effect when injected together with epinephrine (100 mcg./Kg.). Nonadrenolytic vasodilators, such as (−)-isoproterenol (12) and papaverine, do not reduce the toxicity of epinephrine in the rat.

In addition to adrenolysis, the only pharmacological property which is known to protect rats (4) against the lethal effect of epinephrine is that of methacholine, *i.e.*, muscarinic activity. Obviously, the positive readings reported above cannot be due to muscarinic activity, since the active compounds have an antagonistic, not an agonistic action on the muscarinic receptors.

The compounds we tested had weak protective action compared with phentolamine, and as in all cases of weak inhibitory activity, the identification of the type of activity involved is not clear cut. The ED_{50} of phentolamine and those of other adrenolytics (4) are considerably lower than the doses required to reverse the pressor effect of epinephrine. Therefore, if the term "adrenolytic" is applied only to those that reverse the pressor effect of epinephrine, the weakest members of the α-receptor blockers may not be included, although they may show antiepinephrine effect on isolated vessel preparations (rabbit uterus, vas deferens, etc.). Atropine and homatropine were known to reduce the effect of epinephrine on various test objects; our tests confirmed the α-receptor blocking effect of homatropine and identified the optical isomer responsible for the effect of atropine. The chemical similarity of hyoscine and the fact that the racemic mixture, but not the levorotatory isomer, was active strongly suggest that the same mechanism of action is involved. Less is known about the pharmacological actions of compounds I and III which were found weakly active in our tests. However, the chemical relationship with active compounds in our series and the absence of an alternative mechanism of action suggest that the protection against epinephrine toxicity is due to α-receptor blockade.

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