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ON THE PHYSIOLOGICAL DISPOSITION AND POSSIBLE MECHANISM OF THE ANTIHYPERTENSIVE ACTION OF DEBRISOQUIN*

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Abstract—After injection of debrisoquin into rats, the drug was rapidly taken up into several organs, especially the heart. The drug levels then declined rapidly in most tissues for about 4 hr, followed by a period of much slower decline. Low concentrations could be detected in several organs 16 hr after injection. By the use of radioactive debrisoquin, metabolic products of the drug could be found in the heart, liver and intestine 6 hr after drug injection, but at 16 hr, metabolic products were found only in the intestine, urine and feces. About two-thirds of the drug underwent biotransformation. Distinct adrenergic neuronal blocking action, as revealed by inhibition of the physostigmine-induced pressor response, persisted for only about 1½ hr after injection of 5 mg/kg of debrisoquin. This short duration of sympatholytic action in the face of prolonged antihypertensive action in man and the hypertensive rat, coupled with the finding that the low residual amounts of debrisoquin are sufficient to inhibit adrenergic neuronal monoamine oxidase (MAO), suggests that the antihypertensive action of debrisoquin and other neuronal blocking agents may lie, in part, in inhibition of neuronal MAO.

THE CONTINUING search for antihypertensive agents has led to the discovery of a variety of compounds with adrenergic neuronal blocking properties similar to those of bretylium.[1] Debrisoquin (3,4-dihydro-2(1H)-isoquinoline carboxamidine) is another such agent,[2] which has been shown recently to be useful for the treatment of hypertension.[3, 4] Little is known regarding its distribution and fate in the body.

The present paper describes studies on the physiological disposition and metabolism of debrisoquin in the rat. The effect of the drug on amine storage mechanisms is also examined, as the findings on drug disposition and pharmacological actions tend to support the suggestion made earlier that the antihypertensive effect of debrisoquin and other neuronal blocking agents may lie, in part, in their ability to inhibit monoamine oxidase.[5]

MATERIALS AND METHODS

Female Sprague–Dawley rats weighing 160–180 g were used for all experiments except tissue slice experiments. Rats treated with debrisoquin were killed by decapitation; the tissues were quickly excised, trimmed, rinsed in saline and homogenized in

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4 vol. of 0·01 N HCl in a glass homogenizer and debrisoquin content was assayed as described below. For excretion studies, the animals were placed in metabolism cages which permitted the separation of feces and urine.

Debrisoquin was determined by using a modification of a sensitive fluorometric method originally described for the determination of creatine[6] and adapted for the assay of guanethidine[7–9] and guanisoquin.[10] The procedure is based on the finding that guanidines react with ninhydrin in a strongly alkaline medium to form a strongly fluorescent product. A different extraction solvent from that used by the authors mentioned above was used in order to enhance the specificity of the analytical method which is as follows: a 2- or 3-ml aliquot of tissue homogenate is added to a 40-ml glass-stoppered centrifuge tube containing 1 ml of 5 N NaOH, 1·5 g of solid NaCl and 10 ml of a solution composed of 10% butanol and 90% heptane. The tube is shaken for 10 min, centrifuged and an aliquot of the organic phase is transferred to a second tube containing 1 ml NaCl-saturated 0·1 N NaOH. This tube is shaken for 3–5 min to remove any interfering substances which might have been extracted from the tissue homogenate; the tube is centrifuged and an aliquot of the organic phase is transferred to a tube containing 1·5 ml of 0·1 N HCl. This tube is shaken for 1–2 min, centrifuged and 1 ml of the aqueous phase is transferred to a test tube. The fluorophore is formed by successive additions of 0·5 ml of 1% ninhydrin in 95% ethanol and 0·5 ml of a 10% solution of alcoholic KOH. The contents of the tube are thoroughly mixed after each addition. The yellow color which appears upon addition of the alkali disappears after 5 min. The fluorescence produced is maximal between 5 and 10 min after addition of the alkali and fluorescence is measured during this interval with a spectrofluorometer with activation set at 395 mμ and fluorescence at 505 mμ (wavelengths uncalibrated). Fluorescence is proportional to debrisoquin concentration over the range 0·05–5·0 μg/ml.

Tissue blanks gave readings similar to those obtained with reagent blanks. The limit of sensitivity of the method for tissues was 0·05 μg/g where readings about twice that of the blank were obtained. Creatine does not interfere with the method, since it is not extracted by the organic solvent mixture. The drug concentration in unknown samples was determined from the fluorescence reading obtained by carrying known amounts of debrisoquin through the complete procedure. Water recoveries amounted to about 80 per cent. Recovery of the drug added to various tissue homogenates ranged between 92 and 108 per cent of water recoveries.

Evidence for the specificity of the method was obtained by 8-transfer countercurrent distribution, paper chromatography and by comparison of the activation and fluorescence spectra of the ninhydrin-produced fluorophore of authentic debrisoquin and the apparent debrisoquin extracted from hearts of rats drug treated 4 hr previously. The curve obtained after countercurrent distribution between 1 N NaOH and the extraction solvent was the same for apparent and authentic debrisoquin. Ascending paper chromatograms of heart extracts developed with either isopropanol:concentrated ammonium hydroxide:water (20:1:2) or butanol:glacial acetic acid:water (12:3:5) of apparent and authentic debrisoquin were sprayed with pentacyanoaquoferriate or Dragendorf's reagent. A single spot with an R_f value identical to that of authentic debrisoquin was found with both reagents. The activation and fluorescence spectra of the fluorophore formed from authentic and apparent debrisoquin from a variety of tissues were identical.

^{14}C-deprisoquin (labeled at the amidine carbon) was kindly supplied by Hoffman–LaRoche, Inc. and was purified by carrying the compound through the extraction procedure. Unlabeled drug was added to the purified radioactive material to obtain the desired specific activity. For estimation of total radioactivity, tissues were homogenized in ethanol, centrifuged and an aliquot of the supernatant fluid was used for radioactivity measurement in a Beckman liquid scintillation counter. Samples were counted in 10 ml of a solution composed of 5 g of 2,5-diphenyloxazole and 300 mg of 1,4-*bis*-(5-phenyloxazolyl) benzene per l. of toluene. In the case of urine or feces, an aliquot of the aqueous acid phase of the extraction procedure was counted. From 1 to 3 ml of absolute ethanol was added to the counting solution to dissolve aqueous samples.

The arterial blood pressure of rats anesthetized with 1 g/kg of urethane was measured by a Statham pressure transducer connected to a cannula placed in the carotid artery. This preparation was used to study the antagonism by debrisoquin of the pressor response elicited by intravenous injection of physostigmine (20 μg).

Uptake studies *in vitro* were performed on rabbit heart slices. Adult albino rabbits were sacrificed by air embolism and 100–600 mg of heart ventricle slices, 0·3 mm thick, were used for the experiment. The slices were incubated as previously described.[11] Drugs or amines under investigation were added to the beakers after 15 min of preincubation, except as noted. After incubation the slices were removed, rinsed briefly in saline, blotted on filter paper, weighed and assayed for drug or amine

TABLE 1. PHYSIOLOGICAL DISTRIBUTION OF DEBRISOQUIN*

Tissue	Conc of debrisoquin (μg/g wet wt.)						
	30 min	1 hr	2 hr	3 hr	4 hr	8 hr	16 hr
Heart	27·10 ±1·31	18.17 ±1·43	5·66 ±0·41	1·97 ±0·31	0·92 ±0·07	0·32 ±0·07	0·33 ±0·04
Spleen	5·67 ±0·84	3·45 ±0·04	2·62 ±0·17	1·81 ±0·03	1·57 ±0·18	1·06 ±0·21	0·93 ±0·12
Liver	6·62 ±1·69	1·34 ±0·05	1·02 ±0·08	0·48 ±0·02	0·19 ±0·16		
Lung	10·23 ±0·91	5·46 ±0·38	2·02 ±0·15	0·99 ±0·03	0·55 ±0·05	0·30 ±0·05	0·27 ±0·09
Kidney	3·91 ±0·96	1·90 ±0·15	0·84 ±0·04	0·42 ±0·06	0·31 ±0·04	0·16 ±0·04	0·11 ±0·03
Fat	7·31 ±1·49	2·24 ±0·25	0·96 ±0·24	0·61 ±0·06	0·64 ±0·10	0·10 ±0·02	0·11 +0·03
Muscle	2·52 ±0·73	3·56 ±0·35	1·57 ±0·28	2·07 ±0·08	0·15 ±0·04	0·43 ±0·14	0·27 ±0·12
Plasma	0·76 ±0·25	0·50 ±0·06	0·25 ±0·06	0·22 ±0·01	0·25 ±0·03	0·23 ±0·03	0·15 ±0·11

* Rats were treated with debrisoquin (10 mg/kg i.p.) and killed at the times indicated. Debrisoquin content of tissues was assayed as described in Methods. Each value is the mean ±S.E.M. of 5–13 experiments.

content. Metaraminol and *m*-octopamine were measured fluorometrically as described elsewhere.[12] All drug doses and concentrations refer to the free base forms.

RESULTS

Physiological distribution of debrisoquin

Rats were treated with 10 mg/kg of debrisoquin i.p. and then sacrificed at intervals ranging from 30 min to 16 hr. Selected organs were assayed for debrisoquin as described under Methods. The results are shown in Table 1. Debrisoquin was concentrated in several organs, especially heart, as early as 30 min after injection. Tissue levels declined exponentially for about the first 4 hr and the half-life during this period in heart, liver and plasma was about 40 min. The rate of decline of debrisoquin in these tissues lessened after 4 hr, at which time the concentrations were below 1 μg/g in most tissues. Spleen demonstrated a slower rate of decline of drug concentration (half-life about 2·5 hr) earlier after drug administration. Debrisoquin was detectable in most tissues 16 hr after administration. The drug could not be detected in brain at any time. The relation between drug dosage and extent of accumulation in heart and plasma 2 hr after injection was linear over a 10-fold range of drug dosage (Fig. 1). Rats treated with debrisoquin for three consecutive days and sacrificed

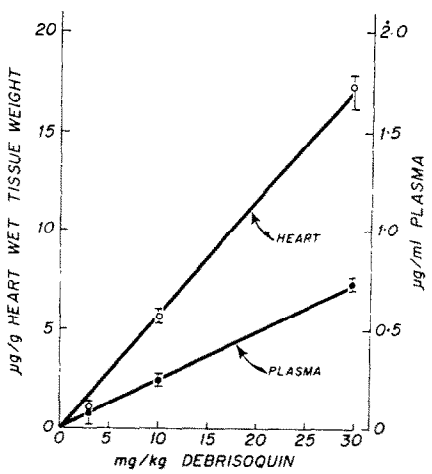

FIG. 1. Concentration of debrisoquin in heart and plasma 2 hr after intraperitoneal administration of various doses of the drug. Figures denote mean ± S.E.

4 hr after the last injection did not show any significant difference in the concentration of the drug in heart, spleen or liver when compared with animals sacrificed 4 hr after a single injection.

Subcellular distribution of debrisoquin

The subcellular distribution of debrisoquin in heart was determined 2 and 5 hr after drug administration. The hearts were homogenized in ice-cold isotonic sucrose and subjected to differential centrifugation at 1000, 10,000 and 100,000 *g*. The results showed that at both times most of the drug was localized in the 1000 *g* and supernatant fractions (Table 2). Only small amounts were found in the 10,000 or 100,000 *g* fractions.

TABLE 2. SUBCELLULAR DISTRIBUTION OF DEBRISOQUIN IN RAT HEART*

Fraction	2 hr (μg ± S.E.)	2 hr (%)	5 hr (μg ± S.E.)	5 hr (%)
1000 *g*	1·246 ± 0·325	34·1	0·672 ± 0·098	50·5
10,000 *g*	0·543 ± 0·241	14·8	0·096 ± 0·010	7·2
100,000 *g*	0·159 ± 0·060	4·3	0·055 ± 0·012	4·1
Supernatant	1·709 ± 0·487	46·7	0·508 ± 0·072	38·2

* Hearts from rats given 10 mg/kg debrisoquin i.p. 2 or 5 hr before killing were homogenized in 4 vol. of isotonic sucrose. The homogenates were centrifuged for 30 min at the indicated forces. Figures denote amounts of drug found in fractions of 1 g of tissue. Four experiments were performed at each time.

Binding of debrisoquin to plasma protein

Rabbit plasma containing 0·2 or 2·0 μg/ml of debrisoquin in dialysis tubing was equilibrated overnight with 0·1 M phosphate buffer, pH 7·4, at 37°. The plasma/buffer concentration ratios found for 0·2 and 2·0 μg/ml were 1·50 and 1·18, respectively, indicating that about 15–30 per cent of the drug is bound to plasma protein.

Metabolism and excretion of debrisoquin

Urine and feces from rats given 10 mg/kg of debrisoquin i.p. were collected for 24 hr and analyzed for drug content. Only one-third of the total amount injected was recovered unchanged. Since the drug disappears rapidly from tissue, most of the drug must be converted to products not measured by the analytical procedure. That this was the case was shown by experiments in which rats were injected with 2 mg ^{14}C-debrisoquin with a specific activity of 2200 cpm/μg and killed after 6 or 16 hr. The quantity of labeled drug available was sufficient for only one experiment at each time. The tissue drug content was determined as before, while the total radioactivity of tissues was obtained using an ethanolic extract. The amount of radioactivity present in the ethanolic tissue extracts was due to debrisoquin plus any metabolites, and an increase in the apparent specific activity (total cpm in tissue/μg debrisoquin in tissue) was an indication of the extent of accumulation of metabolic products. The results obtained are shown in Table 3. The presence of metabolic products of debrisoquin

TABLE 3. PRESENCE OF DEBRISOQUIN METABOLITES IN VARIOUS TISSUES*

Tissue	6 hr (Total cpm/μg deb.)	6 hr (% metabolite)	16 hr (Total cpm/μg deb.)	16 hr (% metabolite)
Heart	2912	29	1578	0
Liver	4312	49	1538	0
Spleen	2006	0	1207	0
Stomach	2299	5	1514	0
Intestine	4047	46	3506	37
Plasma	2016	0	1810	0
Urine	6038	64	6548	67
Feces	—	—	3907	44

* Rats were given 2 mg ^{14}C-debrisoquin (deb.) i.p. and killed at the times indicated. Total radioactivity was measured as described under Methods. Per cent metabolite indicates the percentage of the total (ethanol extractable) radioactivity accounted for by metabolized debrisoquin.

could be detected at 6 hr in heart, liver, intestine and urine, while at 16 hr only intestine, urine and feces contained measurable amounts of debrisoquin metabolites.

Thus, although debrisoquin is extensively metabolized, no metabolic product retaining the amidine group persists in heart, spleen, liver, plasma or stomach.

Urine from these experiments was chromatographed in the two solvent systems described under Methods and sprayed with pentacyanoaquoferriate. The reagent elicited three pink spots, one of which corresponded to authentic debrisoquin. Essentially all of the radioactivity on the chromatograms was confined to these spots.

Uptake in heart slices

The uptake of debrisoquin by rabbit heart slices is shown in Fig. 2. The drug was readily taken up and accumulated by the slices from media containing 10^{-4}, 10^{-6} or

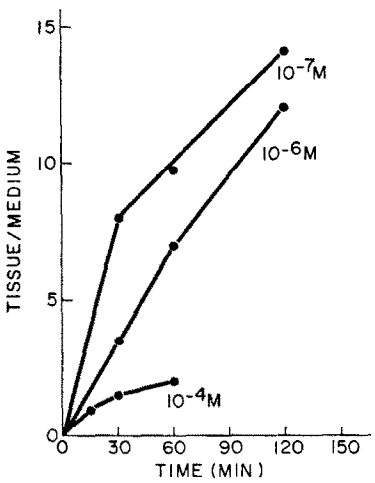

FIG. 2. Accumulation of debrisoquin by rabbit heart slices. The figure denotes tissue/medium concentration ratio after incubation with various concentrations of the drug.

10^{-7} M debrisoquin. The results indicate that at the lower drug concentrations no steady-state distribution between tissue and medium was attained even after 2 hr. Preheating the slices at 55° for 5 min before incubation for 60 min with debrisoquin (10^{-6} M) inhibited uptake by about 50 per cent, while desipramine (10^{-5} M) caused a 44 per cent inhibition of uptake of debrisoquin, and amphetamine (10^{-5} M) depressed accumulation by 29 per cent. Heart slices from rabbits pretreated with reserpine (5 mg/kg i.p.) 16 hr before killing showed no difference from controls in uptake of the drug. Transferring slices to debrisoquin-free media after incubation for 60 min with debrisoquin (10^{-6} M) resulted in a 60 per cent loss of the drug from the slice in 30 min.

Blockade of physostigmine pressor response

The effect of debrisoquin (5 mg/kg i.v.) on the pressor response produced by the intravenous administration of 20 μg physostigmine in rats is shown in Table 4 and Fig. 3. Maximum blockade occurred 60 min after injection, but after 90 min there was little or no inhibition even though analysis at this time showed that debrisoquin was still present in heart in high concentration (8–10 μg/g).

TABLE 4. INHIBITION OF THE PRESSOR RESPONSE TO INTRAVENOUS PHYSOSTIGMINE AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION OF DEBRISOQUIN (5 mg/kg)*

Time after debrisoquin (min)	30	60	90
Per cent blockade of pressor response	20(8–33)	46(41–53)	5(0–16)

* Physostigmine (20 μg) was injected at 30-min intervals before and after debrisoquin treatment. Inhibition was measured by comparing mean arterial blood pressures, defined as diastolic pressure plus one-third of the pulse pressure. Figures denote mean blockade and ranges of six experiments.

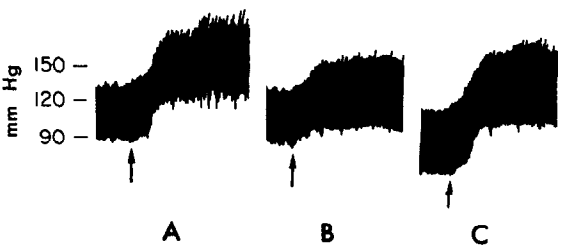

FIG. 3. Effect of debrisoquin (5 mg/kg i.p.) on pressor response to intravenous physostigmine in the urethane-anesthetized rat. A, 20 μg physostigmine before debrisoquin injection; B, physostigmine response 60 min after debrisoquin; C, 90 min after debrisoquin.

Effect of adrenergic neuronal blocking drugs on amine uptake

It is known that both *l*-metaraminol and *l-m*-octopamine accumulate in heart slices by the action of a cocaine-sensitive amine pump localized in the adrenergic neuronal membrane[11] and that the accumulation of *l-m*-octopamine, a substrate of monoamine oxidase (MAO), is enhanced by the blockade of MAO.[11] Table 5 shows the effect of debrisoquin and several other adrenergic neuronal blocking drugs on the uptake of these amines in rabbit heart slices. All of the drugs at a medium concentration of 10^{-4} M greatly inhibited the uptake of metaraminol, indicating that at 10^{-4} M the drugs effectively blocked the membrane amine pump. When slices were incubated in media containing the same drugs at a concentration of $1–2 \times 10^{-6}$ M, there was little or no inhibition of uptake of metaraminol, while accumulation of *l-m*-octopamine was markedly enhanced. The increased accumulation of this amine, as described before,[5] indicates that MAO is inhibited by the neuronal blocking drugs. Additional evidence of this inhibitory action was obtained by use of the *dextro* isomer of *m*-octopamine. It has been shown that the initial rate of accumulation of *d-m*-octopamine is more rapid than that of *l-m*-octopamine due to the fact that the latter is a better MAO substrate.[13] As reported earlier, when *d-m*-octopamine uptake was compared with that of the *l*-isomer, the difference in accumulation between the two isomers was lessened or abolished by low concentrations of bretylium and debrisoquin.[5] Furthermore, under conditions of prior blockade of MAO by pheniprazine (5 mg/kg, 16 hr before the experiment), a maneuver which enhanced the degree of accumulation of *l-m*-octopamine, addition of debrisoquin or bretylium had no further effect.

TABLE 5. EFFECT OF ADRENERGIC NEURONAL BLOCKING DRUGS ON AMINE ACCUMULATION BY RABBIT HEART SLICES*

Drug	Drug concn	Net amine uptake (μg/ml slice water ± S.E.)	
		l-Metaraminol	*l-m*-Octopamine
None		0·38 ± 0·04	0·20 ± 0·02
Debrisoquin	10^{-6}	0·26 ± 0·02	0·97 ± 0·04
Debrisoquin	10^{-4}	0·04 ± 0·02	0·09 ± 0·02
Bretylium	2×10^{-6}	0·35 ± 0·05	0·57 ± 0·02
Bretylium	10^{-4}	0·10 ± 0·01	0·26 ± 0·02
BW 392C60	10^{-6}	0·34 ± 0·02	1·05 ± 0·09
BW 392C60	10^{-4}	0·10 ± 0·02	0·56 ± 0·02
Bethanidine	2×10^{-6}	0·27 ± 0·01	0·49 ± 0·06
Bethanidine	10^{-4}	0·07 ± 0·01	0·22 ± 0·03
Guanisoquin	10^{-6}	0·28 ± 0·03	0·49 ± 0·05
Guanisoquin	10^{-4}	0·01 ± 0·01	0·07 ± 0·02

* Slices were incubated with *l*-metaraminol (0·025 μg/ml) or *l-m*-octopamine (0·2 μg/ml) for 60 min. The blocking drugs were added at the start of the 15-min preincubation period. Net uptake denotes accumulation per ml slice water minus concentration in the medium.

Inhibition of rabbit liver MAO *by neuronal blocking drugs*

Direct evidence that these compounds inhibit MAO was obtained by assaying their action on MAO activity in rabbit liver homogenates as described previously.[13] The concentration of drug required to produce 50 per cent inhibition of enzyme activity, as reflected by the disappearance of *l-m*-octopamine, is shown in Table 6. Debrisoquin and guanisoquin (which is a brominated form of debrisoquin) were the most potent inhibitors among the drugs tested. That bretylium, bethanidine and BW 392C60 inhibit MAO has also been reported elsewhere.[14]

TABLE 6. INHIBITION OF RABBIT LIVER MAO BY ADRENERGIC NEURONAL BLOCKING DRUGS

Compound	Molar concn producing 50% inhibition
Bethanidine	$5{\cdot}0 \times 10^{-4}$
Bretylium	$8{\cdot}6 \times 10^{-5}$
BW 392C60	$3{\cdot}0 \times 10^{-5}$
Debrisoquin	$2{\cdot}0 \times 10^{-6}$
Guanisoquin	$4{\cdot}4 \times 10^{-7}$

DISCUSSION

The localization of debrisoquin in tissues, especially those such as heart or spleen with a high degree of adrenergic innervation, is a property shared by other adrenergic neuronal blocking agents. Accumulation of the administered drug in heart, spleen, liver and kidney has been reported after administration of bretylium,[15] guanethidine,[8] bethanidine[16] and guanisoquin.[10] Although debrisoquin is quickly taken up by these same organs, a rapid initial rate of decline occurs so that the half-life of the drug in most organs during this period is 40 min. After 4 hr, when tissue levels are 1 μg/g or less, the rate of decline is much slower.

The initial rapid rate of disappearance from tissues indicates that the extensive localization seen in tissues early after administration represents binding of a loose, readily reversible nature. As the drug is rapidly and extensively metabolized or excreted and is bound to only a small extent to plasma proteins, levels in the body fall rapidly. The second and persistent phase of the drug decline in tissue indicates that small quantities of debrisoquin are bound in tissues by a second, much firmer bond.

Several other lines of evidence also suggest that a major part of the localization of debrisoquin in tissues may be due to a loose and nonspecific binding. Although debrisoquin is initially concentrated to a greater extent in heart than in any other tissue, examination of the subcellular distribution of the drug in this organ did not reveal specific localization to subcellular fractions, such as the microsomal, commonly associated with neuronal structures. The fact that the relative subcellular distribution of debrisoquin in heart was the same after 2 and 5 hr indicates that the intracellular binding of the drug is relatively nonspecific.

The avid uptake and ready washout of the drug in tissue slices parallel the high initial accumulation and rapid rate of disappearance of the drug *in vivo*. The only partial impairment of accumulation by heat denaturation, desipramine and amphetamine suggests that uptake may occur by more than one process in a manner similar to that reported for guanethidine.[8] The linearity of uptake by heart slices with time as well as the linear relationship between dose and accumulation *in vivo* provides further evidence that this process represents a loose and non-specific binding to cellular structures.

Debrisoquin is extensively metabolized in the rat, only about one-third of the injected dose appearing unchanged in the urine and feces. The two metabolic products observed in the urine retain the amidine grouping as evidenced by the retention of radioactivity in the products seen after paper chromatography of urine and the positive nature of the reaction with the pentacyanoaquoferriate reagent.

These results with debrisoquin are in agreement with findings reported elsewhere[4] and are also similar to those found with guanethidine.[7, 17] The absence of a high concentration of detectable metabolites in the heart and most other organs suggests that the cardiovascular effects of the drug are produced by unchanged debrisoquin.

The pressor response produced by small doses of physostigmine in the rat has been shown to result from centrally mediated sympathetic stimulation[18] and has been used to investigate the adrenergic blocking action of bretylium and guanethidine.[19, 20] Although debrisoquin initially antagonized the pressor response, this action was only short lived, disappearing after about 1·5 hr. This short duration of action contrasts with the results reported for bretylium, where the pressor response was blocked for 4 or 5 hr.[19]

This short duration of the definite sympatholytic effect of debrisoquin contrasts sharply with the ability of the drug to lower the blood pressure of hypertensive rats for periods of up to 24 hr after drug administration.[2] As there is no continued accumulation of debrisoquin or its metabolic products in the body when the drug is given daily, it would seem that the initial marked sympatholytic properties of debrisoquin may not be the main or sole mechanism involved in the antihypertensive action of the drug.

Although other neuronal blocking drugs also show considerable initial localization in tissues of high adrenergic innervation, certain of these drugs also disappear rapidly

from tissues and the duration of neuronal blockade is correspondingly short. For example it has been demonstrated that 2-(4-benzoyl-2, 6-dimethylphenoxyl)-ethyl trimethylammonium has a high intrinsic neuronal blocking activity and accumulates in adrenergic neurones, but lacks the persistence of retention of bretylium and thus produces very little adrenergic neuronal blocking in man.[21] In a similar manner, the ease of washout of debrisoquin found *in vitro* and its rapid removal from tissues *in vivo* are evidence that these actions may prevent any persistence of the true neuronal blocking activity of this drug. Furthermore, the clinical reports concerning debrisoquin indicate that the onset of hypotension occurs within 8–12 hr after administration and, after a single dose, is terminated 24 hr later.[3, 22] This length of action in man as well as in the rat and the short duration of blockade of the physostigmine-induced pressor response argue further against neuronal blockade as the principal mode of clinical antihypertensive action of debrisoquin.

In a previous communication,[5] the ability of debrisoquin and bretylium to affect amine uptake and inhibit MAO was reported. The system utilized allows differentiation between amine uptake by a relatively nonspecific neuronal membrane amine pump and accumulation by an intracellular storage mechanism.[11] The same conclusion may now be reached in the case of still other adrenergic neuronal blocking drugs. Thus, accumulation of metaraminol is inhibited by high concentrations (10^{-4} M) of these drugs, while at low concentrations (10^{-6}–10^{-7}) the compounds cause little inhibition of the membrane amine pump, but inhibit MAO as evidenced by the increased accumulation of *l-m*-octopamine, a substrate of this enzyme. Similar results and interpretation have been presented in studies on the actions of bretylium on norepinephrine accumulation in reserpinized guinea pig heart.[23]

Further evidence that these drugs promote accumulation of an MAO substrate by inhibiting MAO is shown by the finding that no further enhancement of accumulation of *l-m*-octopamine by debrisoquin is seen after prior treatment with a classical MAO inhibitor. Direct evidence that debrosoquin and related drugs are MAO inhibitors is seen in their action *in vitro* on rabbit liver preparations. It should be noted that guanethidine, which acts as a neuronal blocking drug for only a short time after injection,[20] is not an MAO inhibitor and is, in fact, a depleter of norepinephrine. Additional evidence that debrisoquin blocks MAO *in vivo* is seen in experiments showing that pretreatment of animals with the drug blocks the disappearance of norepinephrine caused by reserpine or guanethidine,[2] an action similar to that of classical MAO inhibitors.

The mechanism of action whereby MAO inhibitors exert an antihypertensive action is not understood. However, it seems that a relationship does exist between the ability of these drugs to decrease blood pressure and their MAO inhibiting property.[24] The MAO inhibitory action of debrisoquin, coupled with the fact that small quantities of the drug are present for prolonged periods in several tissues, indicates that the antihypertensive mechanism may be, at least in part, that of the classical MAO inhibitors. This would be consistent with the duration of the antihypertensive action of the drug being more prolonged than the duration of the observable adrenergic neuronal blocking action. Thus, after chronic treatment of hypertensive rats, blood pressure did not revert to pre-drug levels until up to 3 days after cessation of drug administration.[2] This residual antihypertensive action seems to be characteristic of the MAO inhibitors.

Indication that the low but persistent levels of debrisoquin in rat tissues are sufficient to inhibit MAO is seen by a comparison of the concentration of the drug found in tissues 16 hr after injection and that required to inhibit MAO as evidenced by enhancement of *l-m*-octopamine uptake by heart slices. Thus, the concentration of debrisoquin present in heart 16 hr after administration of 10 mg/kg approximates the amount present in heart slices after 60 min of incubation of slices with debrisoquin (10^{-7} M), a concentration sufficient to enhance *l-m*-octopamine accumulation by the slice. Thus it seems likely that repeated doses would be sufficient to maintain these low concentrations in tissues of high affinity. Consequently, inhibition of MAO could be adequately sustained throughout the period of administration. It is therefore possible that the antihypertensive action of the adrenergic neuronal blocking agents may not be due solely to their strictly neuronal blocking properties but may also stem, in part, from their ability to persistently inhibit MAO in adrenergically innervated tissues.

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