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INFLUENCE OF DEBRISOQUIN (DECLINAX) ON ADRENERGIC FUNCTION AND NOREPINEPHRINE METABOLISM

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Abstract—The influence of debrisoquin on the uptake, depletion and metabolism of ^{3}H-norepinephrine has been investigated in rat heart and brain tissue. Debrisoquin noncompetitively inhibits the uptake of ^{3}H-norepinephrine at the neuronal membrane. Debrisoquin also decreases the ability of amine storage vesicles to retain norepinephrine and, therefore, may decrease the amount of neurotransmitter which is available for release by nerve stimulation. The monoamine oxidase inhibition produced by debrisoquin prevents the intraneuronal metabolism of the free cytoplasmic pool of norepinephrine and, hence, there is little tissue depletion of norepinephrine.

PHARMACOLOGICAL studies of the antihypertensive agent, debrisoquin sulfate (Declinax), have shown that this drug alters a number of sympathetic functions. In dogs, debrisoquin initiated an interval of hypertension followed by a prolonged period of hypotension, while in cats the drug blocked preganglionic, but not tyramine, stimulation of the nictitating membranes.[1] Debrisoquin also potentiated the effects of administered norepinephrine in cats[1] and humans.[2]

In rabbit heart tissue slices, debrisoquin (10^{-6}M) promoted the accumulation of octopamine, a monoamine oxidase substrate, but at higher doses (10^{-4}M) blocked the accumulation of both octopamine and l-metaraminol.[3] These effects on amine accumulation have suggested that debrisoquin inhibited both the neuronal membrane amine pump and monoamine oxidase (MAO). Pocelinko *et al.*[4] have observed a decreased excretion of 3-methoxy-4-hydroxymandelic acid in the urine of debrisoquin-treated human subjects, suggesting that the drug inhibits monoamine oxidase.

In human blood platelets, debrisoquin inhibited tyramine oxidation noncompetitively, diminished the accumulation of serotonin competitively, and depleted serotonin stores.[5]

This study describes additional effects of debrisoquin upon the uptake, accumulation and storage of norepinephrine by adrenergic neurons in rat heart and brain tissues.

METHODS

Male Royal Hart rats, weighing 190–210 g, were used in all experiments.

LD-norepinephrine-7-^{3}H (sp. act., 9 c/m-mole) was obtained from New England Nuclear Corp. Cocaine hydrochloride was procured from Merck & Company, Inc.

Tritium was assayed in a liquid scintillation spectrometer. A *p*-dioxane (spectro-

quality) counting medium containing 0·6% butyl-PBD and 6·3% naphthalene (recrystallized) was employed. The counting efficiency was determined with an external ^{133}Ba standard.

The effect of debrisoquin on ^{3}H-norepinephrine accumulation

In heart. Various doses of debrisoquin sulfate were given intraperitoneally (i.p.) in 0·5 ml of water. Thirty min after the drug administration, 10 μc of ^{3}H-norepinephrine was injected intravenously (i.v.) into each rat. The animals were sacrificed 15 min later. The hearts were removed, homogenized (glass-dual homogenizers) in 5 ml of 0·4 N perchloric acid, frozen, thawed and centrifuged for 15 min at 400 *g*. Aliquots (4 ml) of the supernatant were assayed for ^{3}H-norepinephrine, ^{3}H-normetanephrine, and the ^{3}H-deaminated catechols by methods described by Kopin *et al.*[6]

In brain. Thirty min after the i.p. injection of debrisoquin (25 mg/kg), 1·0 μc of ^{3}H-norepinephrine was injected intracisternally and the rats were sacrificed 6 min later. The procedures for performing the intracisternal injections and the processing of the brains after their removal have been described by Schanberg *et al.*[7,8]

In isolated perfused hearts. Isolated rat hearts were perfused with Krebs–Ringer bicarbonate buffer as described elsewhere.[9] To demonstrate the effects of debrisoquin or cocaine on ^{3}H-norepinephrine uptake, hearts were perfused with media containing either debrisoquin (10^{-6}M) or cocaine (10^{-6}M) for 10 min, followed by a 1-min perfusion with media containing 50 μc/l. ^{3}H-norepinephrine (sp. act., 170 μc/μmole), as well as either debrisoquin or cocaine, A 2-min wash-out with norepinephrine free media terminated the perfusion experiment.

The influence of debrisoquin on norepinephrine uptake kinetics was determined with isolated rat hearts in experiments similar to those just described except the perfusate ^{3}H-norepinephrine specific activity was adjusted to 425, 85 or 43 μc/μmole by the manipulation of unlabeled norepinephrine concentrations.

One- or 2-min perfusions with norepinephrine were employed in the above experiments since these time intervals are known to provide amine uptake rates which closely approximate initial rates of uptake.[10]

The accumulation of ^{3}H-norepinephrine by isolated hearts in the presence of debrisoquin was determined by perfusing the hearts for various time intervals (5–40 min) with ^{3}H-norepinephrine (sp. act., 5 mc/μmole). Debrisoquin (10^{-6}M or 10^{-5}M) was included in both the norepinephrine free and the norepinephrine supplemented media. The norepinephrine free perfusion periods were varied with each of the ^{3}H-norepinephrine perfusion times and are listed in Table 1. The hearts were perfused with Krebs–Ringer buffer for 2 min after the norepinephrine perfusion.

All hearts were homogenized in 5 ml 0·4 N perchloric acid, frozen, thawed, centrifuged (400 *g*), and the supernatant assayed for tritium. Since greater than 95 per cent of the tritium in isolated hearts perfused with ^{3}H-norepinephrine is associated with norepinephrine,[11,12] all tritium was calculated as norepinephrine.

The effects of debrisoquin on ^{3}H-norepinephrine depletion

In heart. Rats were injected intravenously with 10 μc ^{3}H-norepinephrine and 1 hr later they were given either debrisoquin (25 mg/kg) or saline intraperitoneally Two hr after drug administration, the rats were sacrificed and the hearts analyzed for ^{3}H-norepinephrine, ^{3}H-normetanephrine, and ^{3}H-deaminated catechols.[6]

TABLE 1. PERFUSION TIMES UTILIZED IN DETERMINING THE EFFECTS OF DEBRISOQUIN ON THE ACCUMULATION OF NOREPINEPHRINE BY ISOLATED RAT HEARTS*

Krebs–Ringer (min)	Krebs–Ringer with norepinephrine (min)
19	2
17·5	5
15	10
10	20
5	30
0	40

* Debrisoquin was included in both perfusion media.

In brain. Ninety min after the intracisternal injection of 10μc of ^{3}H-norepinephrine, debrisoquin (25 mg/kg) was given intraperitoneally and the brains removed for analysis 3 hr later.

RESULTS

The effects of debrisoquin on the accumulation *in vivo* and metabolism of ^{3}H-norepinephrine by rat heart tissue are illustrated in Fig. 1. The administration of low doses (1 mg/kg) of debrisoquin prior to the injection of ^{3}H-norepinephrine causes a substantial decrease in the concentration of ^{3}H-deaminated metabolites and an increase in ^{3}H-normetanephrine concentration. With doses of 5 to 50 mg/kg, debrisoquin also inhibits the accumulation of ^{3}H-norepinephrine.

Debrisoquin (25 mg/kg) given 1 hr after ^{3}H-norepinephrine (sufficient time to permit amine uptake and storage) causes a 24 per cent reduction in heart tissue ^{3}H-norepinephrine and deaminated catechols and a 20 per cent increase in ^{3}H-normetanephrine (Table 2) after 2 hr.

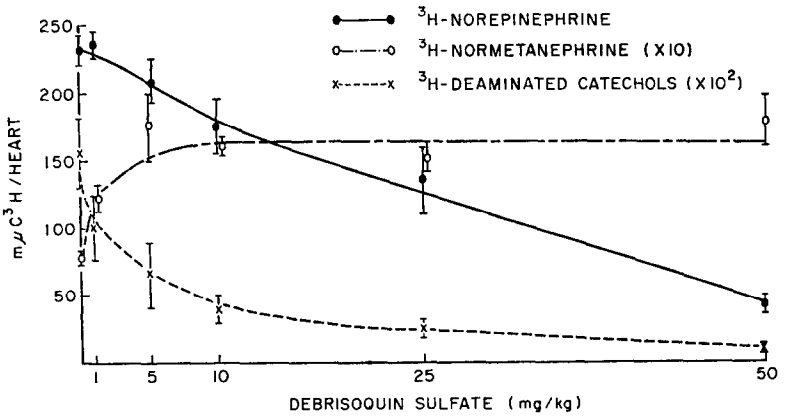

FIG. 1. Effect of debrisoquin on the accumulation *in vivo* and metabolism of ^{3}H-Norepinephrine by rat hearts. Rats were given debrisoquin or saline 30 min before the intravenous administration of ^{3}H-norepinephrine (10μc). The animals were sacrificed 15 min after the ^{3}H-norepinephrine administration. Each value is the mean of six hearts $\pm$ S.E.M.

TABLE 2. INFLUENCE OF DEBRISOQUIN ON ^{3}H-NOREPINEPHRINE DEPLETION FROM RAT HEART TISSUE*

^{3}H-norepinephrine (10 μc, i. v.) —60 min→ Debrisoquin (25 mg/kg, i.p.) —120 min→ Sacrifice

^{3}H-metabolites	Control	Debrisoquin	Change (%)	P
Norepinephrine	79·1 ± 4·8	59·8 ± 3·7	−24	< 0·05
Normetanephrine	0·38 ± 0·03	0·46 ± 0·03	20	> 0·05
Deaminated catechols	0·48 ± 0·01	0·37 ± 0·03	−24	< 0·01

* Values are expressed as millimicrocuries of tritium per heart and are the mean ± S.E.M. of at least seven animals.

TABLE 3. EFFECT OF DEBRISOQUIN ON THE ACCUMULATION OF ^{3}H-NOREPINEPHRINE BY RAT BRAIN TISSUE*

Debrisoquin (25 mg/kg, i. p.) —30 min→ ^{3}H-norepinephrine (1·0 μc, i. c.) —6 min→ Sacrifice

^{3}H-metabolites	Control	Debrisoquin	Change (%)	P
Norepinephrine	366·79 ± 27·29	375·01 ± 33·85		> 0·05
Normetanephrine	130·99 ± 10·51	191·35 ± 15·70	46	< 0·05
Deaminated catechols	42·47 ± 3·91	14·81 ± 1·48	−65	< 0·001

*Values are expressed as millimicrocuries of tritium per gram of brain and are the mean ± S.E.M. of seven animals.

TABLE 4. EFFECTS OF DEBRISOQUIN AND COCAINE ON ^{3}H-NOREPINEPHRINE UPTAKE BY PERFUSED RAT HEARTS*

Drug	mμc ^{3}H heart	Change (%)	P
Control	25·1 ± 2·03		
Debrisoquin (10^{-6}M)	10·4 ± 0·37	−58	< 0·001
Cocaine (10^{-6}M)	8·2 ± 0·67	−68	< 0·001

*Hearts were perfused for 10 min with perfusate containing debrisoquin or cocaine, followed by perfusion with norepinephrine (50 ng/ml). The hearts were perfused for a final 2-min period with a norepinephrine-free medium. Each value is the mean of six hearts ± S.E.M.

Debrisoquin (25 mg/kg) pretreatment affects the metabolism of intracisternally injected ^{3}H-norepinephrine in rat brains (Table 3) similar to those effects described in the heart tissue at low drug doses (1mg/kg). The drug increases the brain levels of ^{3}H-normetanephrine, decreases the concentrations of ^{3}H-deaminated catechols, but has no effect on ^{3}H-norepinephrine accumulation. Debrisoquin (25 mg/kg) given 90 min after the intracisternal injection of ^{3}H-norepinephrine does not deplete or influence the metabolism of the labeled amine.

When debrisoquin is perfused through isolated rat hearts at a concentration of 10^{-6}M, the accumulation of ^{3}H-norepinephrine is inhibited by 60 per cent (Table 4). Debrisoquin is nearly as potent as cocaine in preventing the accumulation of norepinephrine. An analysis of norepinephrine uptake kinetics in the presence of debrisoquin indicates that this drug is a noncompetitive inhibitor of amine uptake (Fig. 2).

Although debrisoquin is not very effective in depleting tissue norepinephrine, the blocking of preganglionic stimulation,[1] as well as the depletion of serotonin from blood platelets by this drug,[5] suggest that debrisoquin may alter the amine storage capacity of intraneuronal vesicles.

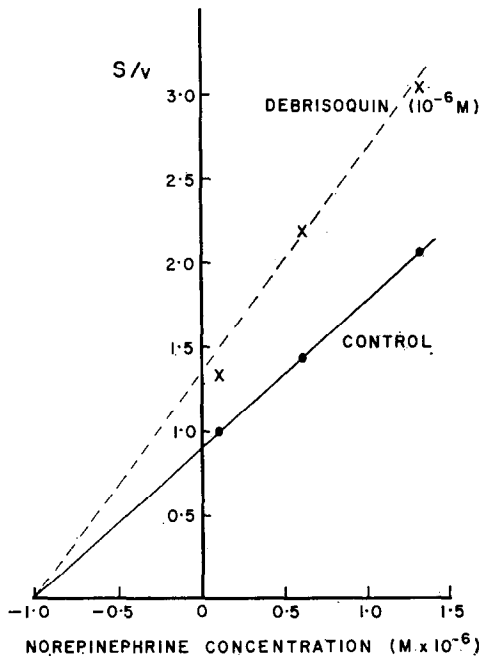

FIG. 2. Influence of debrisoquin on the Michaelis–Menten kinetics of norepinephrine uptake by isolated perfused hearts. Rat hearts were perfused for 10 min with or without debrisoquin (10^{-6}M), followed by a 2-min perfusion with norepinephrine (20, 100 or 220 ng/ml). The perfusions were concluded with a 2-min washout of norepinephrine free media. S = norepinephrine concentration in the perfusate; v = initial rate of uptake (see Methods); K_m = intercept on the abscissa; $1/V_{max}$ = slope.

Iversen *et al.* have suggested that norepinephrine accumulation during short perfusions reflects the rate of amine transport across the neuronal membrane, while accumulation during long norepinephrine perfusions is indicative of the intraneuronal amine storage capacity.[12] In order to reveal effects of debrisoquin on norepinephrine storage in sympathetic neurons, rat hearts were perfused for various time intervals with ^{3}H-norepinephrine.

The effects of debrisoquin (10^{-6} and 10^{-5}M) on norepinephrine accumulation during various time intervals (5–40 min) are illustrated in Fig. 3. When present at a

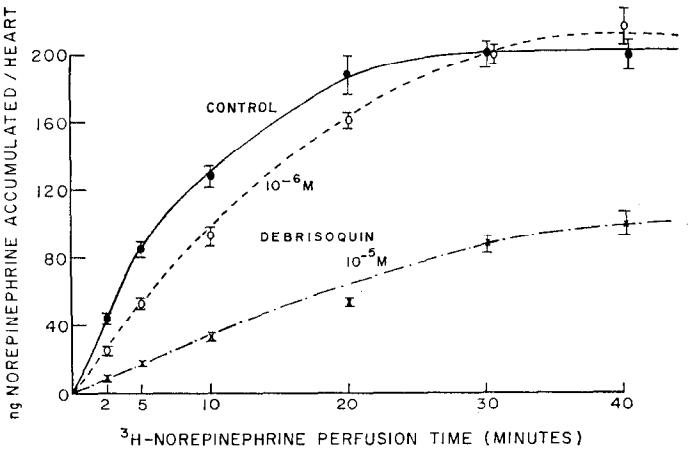

FIG. 3. Influences of debrisoquin on norepinephrine accumulation in isolated perfused rat hearts. Perfusion times are listed in Table 1. The ^{3}H-norepinephrine perfusate contained 200 ng/ml of ^{3}H-norepinephrine. Each value is the mean of six hearts ±S.E.M.

concentration of 10^{-6}M, debrisoquin decreases the initial rate of amine uptake but does not lower the capacity of heart tissue to retain norepinephrine. At concentrations of 10^{-5}M, however, debrisoquin decreases both the initial rate of amine uptake, as well as the capacity to retain norepinephrine.

DISCUSSION

The re-uptake of norepinephrine into sympathetic nerve endings is known to be the major route by which this neurotransmitter is inactivated. Compounds, such as cocaine, which block the amine uptake process have been observed to potentiate organ response to nerve stimulation and administered norepinephrine.[13–16] Thus, the period of hypertension which follows debrisoquin administration to dogs[1] and humans[2] may be because of the inhibition of the amine uptake process by this drug.

Since debrisoquin is a monoamine oxidase inhibitor,[3–5] norepinephrine released by this drug would likely leave the neuron in a physiologically active form and augment the initial potentiation of sympathetic function.

While debrisoquin does not inhibit the accumulation of intracisternally administered ^{3}H-norepinephrine in rat brains, it does cause a shift in amine metabolism similar to that found in rat hearts after low (1 mg/kg) doses of debrisoquin. Debrisoquin, or an active metabolite, may cross the blood brain barrier very slowly so that the extraneuronal concentration of the drug is insufficient to block the amine uptake mechanism but intraneuronal drug levels may rise sufficiently to block monoamine oxidase.

Giachetti and Shore[3] have suggested that debrisoquin is taken up and concentrated within sympathetic neurons. The ability of adrenergic neurons to concentrate the drug would provide an explanation for the intraneuronal debrisoquin-induced changes in monoamine oxidase activity even though no effect on norepinephrine uptake is noted.

The observations in this study indicate that debrisoquin interferes with norepinephrine transport across the neuronal membrane and with retention of the neurotransmitter within the storage vesicles. The same dual action has been reported for the hypotensive agent, guanethidine.[17–19]

Since increasing evidence suggests that only norepinephrine, bound in storage vesicles, is available for release by nerve stimulation,[20–21] the decrease in norepinephrine storage capacity noted after debrisoquin treatment may be largely responsible for blocking the preganglionic stimulation and for the hypotensive phase of the cardiovascular response to this drug. Although there is relatively little tissue depletion of amines by debrisoquin as compared with the depleting action of guanethidine,[1] the potent monoamine oxidase inhibition produced by debrisoquin would permit the intraneuronal release of norepinephrine from storage vesicles without its subsequent oxidation and exodus from the neuron as an inactive metabolite.

With a debrisoquin-induced shift of norepinephrine from vesicular to free cytoplasmic sites, a supersensitivity to tyramine administration might be anticipated.[22] Observations depicting a supersensitivity to tyramine by debrisoquin-treated patients have been reported.[4]

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