Interaction of Cyclazocine and the Sympathetic Nervous System

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Abstract □ Cyclazocine, a benzomorphan derivative narcotic agonist–antagonist, reduced the uptake of tritiated norepinephrine and reduced the recovery of [^{3}H]3,4-dihydroxymandelic acid, but did not significantly alter the recovery of [^{3}H]normetanephrine in the rat heart *in vivo*. Cyclazocine also reduced endogenous levels of norepinephrine in the rat heart. Comparatively, desipramine reduced the uptake of [^{3}H]norepinephrine, the recovery of [^{3}H]3,4-dihydroxymandelic acid, and the recovery of [^{3}H]normetanephrine in the rat heart *in vivo*. Further, cyclazocine added to the perfusion medium or administered systemically reduced the uptake of radiolabeled norepinephrine by the isolated rat heart. The cyclazocine-induced decrease in the accumulation of [^{3}H]norepinephrine in the rat heart *in vivo* and *in vitro* presumably is due to an alteration of sympathetic function through the inhibition of neuronal uptake. It is further suggested that cyclazocine has other actions on the sympathetic nervous system, such as promoting neurotransmitter release.

Keyphrases □ Cyclazocine—interaction with the sympathetic nervous system □ Sympathetic nervous system—interaction with cyclazocine

Cyclazocine was first synthesized in the search for nonaddicting analgesics. Although cyclazocine is an effective analgesic in animals (1) and humans (2, 3), it has been shown to be a potent narcotic antagonist (4, 5). Consequently, it has been employed in the treatment of narcotic addiction (6–9). In these patients, a cyclazocine-induced euphorigenic and antidepressant action was noticed (7). Subsequently, the clinical evaluation of cyclazocine in depressed patients was reported (10, 11). It was concluded that cyclazocine exhibited a clinical antidepressant action with a range of activity very similar to imipramine.

Support for clinical data (10) was found by demonstrating that the cyclazocine-induced electroencephalogram changes were similar to those induced by imipramine in depressed patients. Likewise, electroencephalogram changes induced by cyclazocine and imipramine have been reported to be similar in the rabbit (12, 13).

Tricyclic antidepressants (imipramine and related compounds) have been shown to potentiate certain cardiovascular responses to norepinephrine in cats (14) and humans (15), presumably by preventing the uptake of norepinephrine by sympathetic neuronal structures (16, 17). Likewise, cyclazocine potentiates certain actions of norepinephrine *in vivo* and *in vitro* (18).

Since these studies suggest that cyclazocine may possess pharmacological actions similar to tricyclic antidepressants, the possibility that cyclazocine may alter sympathetic nervous system function was investigated.

EXPERIMENTAL

Cannulation of Vessels—The animals (male Sprague-Dawley rats, 200–350 g) were anesthetized with ether, and the right femoral vein was cannulated to facilitate intravenous drug injections. Each injection was washed into the vein with a 0.2-ml solution of normal saline.

Perfusion of the Rat Heart—All rats were given 1000 U ip of heparin 5 min prior to decapitation to reduce intravascular clotting. The hearts were removed and perfused by the Langendorff technique (19). After a 10-min perfusion of amine-free modified Krebs-bicarbonate solution at 60 mm Hg, the hearts were perfused until a constant volume was reached (~10 min), with modified Krebs-bicarbonate solution containing 3.3 nCi of [^{3}H]norepinephrine, diluted with nonradioactive norepinephrine to a final concentration of 10 ng/ml. Following the amine perfusion, the extraneuronal norepinephrine was removed by a 2-min wash with amine-free medium. This washing technique has been reported to remove 95% of the extracellular norepinephrine (20). The heart was removed from the perfusion apparatus, blotted, weighed, and assayed for [^{3}H]norepinephrine.

Each liter of bathing medium, continuously bubbled with a mixture of 5% carbon dioxide in oxygen, contained (m*M*): NaCl (118); $NaHCO_3$ (25); KCl (4.75); $MgSO_4$ (1.19); KH_2PO_4 (1.19); $CaCl_2 \cdot 2H_2O$ (2.5); glucose, 2 g; disodium edetate, 0.010 g.

Norepinephrine and Metabolite Assay—The isolation procedure for radioactive and endogenous norepinephrine and its metabolites was carried out by the use of ion-exchange resins and alumina (21).

Norepinephrine was made to fluoresce by the trihydroxyindole technique (22). The fluorescence was read on a spectrofluorometer[1]. Efficiency of the assay was determined by the use of a standard carried completely through the assay. Reagent fluorescence was determined after carrying each reagent used for elution through the assay. Mean recovery rates were: norepinephrine, 90%; normetanephrine, 72%; dihydroxymandelic acid, 85%.

For estimation of the tritiated catecholamine and the tritiated metabolites, 1 ml of the appropriate eluate containing the tritiated norepinephrine or metabolite was added to 10 ml of a commercial counting cocktail[2] and counted in a liquid scintillation counter[3]. Efficiency and quench were determined by using a tritium external standard.

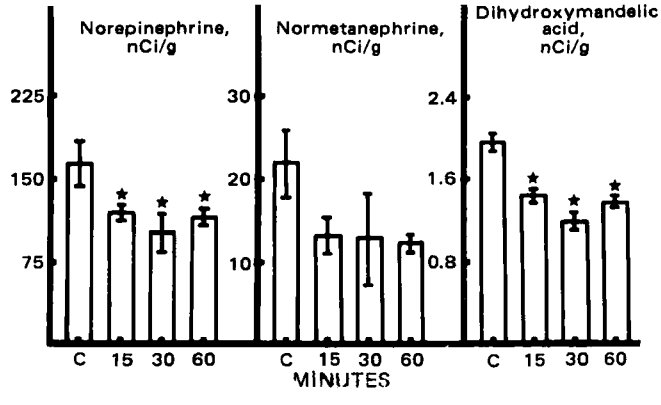

Figure 1—*The effect of cyclazocine on the uptake and metabolism of tritiated norepinephrine in rat hearts* in vivo. *Rats were given cyclazocaine (5 mg/kg iv) 15, 30, and 60 min prior to an infection of 10 μCi iv % tritiated norepinephrine. Control animals received an intravenous injection of saline. The hearts were removed 30 min later and assayed for the radiolabeled compounds, norepinephrine, normetanephrine, and 3,4-dihyroxymandelic acid. All values represent the means (horizontal bar) ± SEM (vertical bar) from four rats. Key: (*), postcyclazocine values significantly different from control values (C) at* $p < 0.05$.

[1] Aminco-Bowman model XLS IA M2.
[2] Aquasol, New England Nuclear Corp., Boston, Mass.
[3] Beckman model 230.

0022-3549/83/0200-0151$01.00/0

Table I—Effect of Cyclazocine on Endogenous Heart Norepinephrine in the Rat [a]

Time, hr	Heart Norepinephrine, μg/g			
	Cyclazocine, mg/kg			
	5	10	20	40
Control	0.87 ± 0.07	0.91 ± 0.03	0.93 ± 0.03	0.95 ± 0.05
1	0.79 ± 0.09	0.79 ± 0.02[b]	0.70 ± 0.08[b]	0.80 ± 0.07
2	0.87 ± 0.04	0.70 ± 0.02[b]	0.65 ± 0.05[b]	0.69 ± 0.03[b]
4	0.88 ± 0.08	0.57 ± 0.01[b]	0.83 ± 0.04	0.79 ± 0.11

[a] $n = 4$; all values represent the mean ± *SEM*. [b] $p < 0.05$.

Drugs and Solutions—Cyclazocine[4] was dissolved in acidic aqueous solution prior to use. In the perfused heart studies, cyclazocine was dissolved using 10% polysorbate 80[5] as a cosolvent in water and added directly to the bathing medium. The medium containing no cyclazocine received an equivalent amount of polysorbate 80.

[7-^{3}H]DL-Norepinephrine (5.0 or 15.8 Ci/m*M*)[6] solutions were made by diluting a proportion of the commercial stock solution with 0.1 *N* HCl containing 0.85% NaCl and norepinephrine bitartrate[7] to the desired specific activity.

Desipramine hydrochloride[8] was dissolved in double distilled water containing 0.85% NaCl.

Statistical Analysis—The data presented as means ± *SE* were analyzed with a randomized complete block analysis of variance with Duncan's new multiple range test (23). Values of $p < 0.05$ were considered significant.

RESULTS

Influence of Cyclazocine on the Uptake and Metabolism of [^{3}H]norepinephrine *In Vivo*—Cyclazocine significantly altered the uptake and metabolism of tritiated norepinephrine in the rat heart. After an intravenous injection of cyclazocine (15, 30, and 60 min) (5 mg/kg) the uptake of [^{3}H]norepinephrine (10 μCi) was significantly reduced (Fig. 1). Cyclazocine did not significantly alter the catechol-*O*-methyltransferase metabolite, normetanephrine, although there appeared to be some reduction. The monoamine oxidase metabolite, 3,4-dihyroxymandelic acid, was significantly reduced by cyclazocine at all three time periods measured.

An evaluation was made of the effect of three doses of cyclazocine, 2.5, 5.0, or 10 mg/kg iv on the uptake of the tritiated amine at the 30-min interval. The uptake of [^{3}H]norepinephrine was significantly reduced at doses of 2.5, 5.0, and 10 mg/kg (Fig. 2).

Effect of Desipramine on the Uptake and Metabolism of [^{3}H]-Norepinephrine *In Vivo*—Intravenous desipramine (5 mg/kg) significantly reduced the uptake of [^{3}H]norepinephrine and altered the formation of radiolabeled dihydroxymandelic acid at all time periods tested (Fig. 3). Unlike cyclazocine, desipramine significantly altered tritiated normetanephrine at all time periods measured.

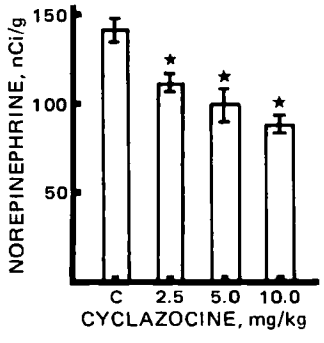

Figure 2—*The effect of various doses of cyclazocine on the uptake of tritiated norepinephrine in the rat heart* in vivo. *Rats were given cyclazocine 2.5, 5.0, or 10.0 mg/kg 30 min prior to an injection of 10 μCi iv of tritiated norepinephrine. Control animals received an intravenous injection of saline. The hearts were removed 30 min later and assayed for tritiated norepinephrine. All values represent the mean (horizontal bar) ± SEM (vertical bar) from four rats. Key: (*), postcyclazocine values significantly different from control values (C) at* $p < 0.05$.

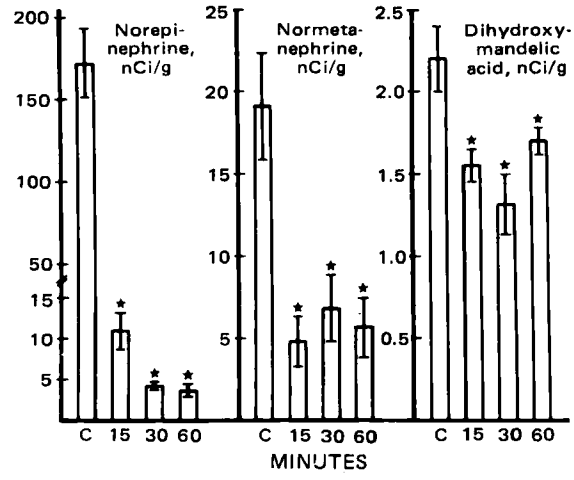

Figure 3—*The effect of desipramine on the uptake and metabolism of tritiated norepinephrine in rat hearts* in vivo. *Rats were given desipramine (5 mg/kg iv) 15, 30, and 60 min prior to an injection of 10 μCi of [^{3}H]norepinephrine. Control animals received an intravenous injection of saline. The hearts were removed 30 min later and assayed for the radiolabeled compounds, norepinephrine, normetanephrine, and 3,4-dihydroxymandelic acid. All values represent the mean (horizontal bar) ± SEM (vertical bar) from four rats. Key: (*), postdesipramine values significantly different from control values (C) at* $p < 0.05$.

Influence of Cyclazocine on the Uptake of Tritiated Norepinephrine *In Vitro*—The intraperitoneal injection of cyclazocine, 2.5, 5, or 10 mg/kg, 1 hr before the removal of the rat heart and subsequent perfusion with radiolabeled norepinephrine significantly reduced the uptake of the tritiated norepinephrine (Fig. 4). Cyclazocine added directly to the bathing medium in concentrations of 10^{-5} and 10^{-4} *M*, likewise, significantly reduced the uptake of [^{3}H]norepinephrine by the isolated perfused rat heart (Fig. 5).

Effect of Cyclazocine on Heart Endogenous Norepinephrine—Table I shows the influence of cyclazocine, *5, 10, 20, or 40 mg/kg ip on rat* heart norepinephrine 1, 2, or 4 hr after cyclazocine. Cyclazocine significantly reduced rat heart norepinephrine levels at doses of 10, 20, and 40 mg/kg, but did not alter the levels at 5 mg/kg.

DISCUSSION

These results support the hypothesis that cyclazocine produces alterations in sympathetic nervous system function by virtue of changes in uptake processes at adrenergic nerve endings.

A reduced recovery of [^{3}H]norepinephrine in rat heart *in vivo* produced by cyclazocine could be related to an altered uptake of norepinephrine

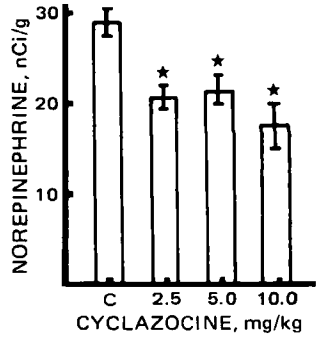

Figure 4—*The effect of systemic cyclazocine on the uptake of tritiated norepinephrine by the isolated perfused rat heart. Rats were given cyclazocine 2.5, 5.0, or 10.0 mg/kg ip 1 hr prior to removal of the heart. Control rats received an intraperitoneal injection of saline. The hearts were perfused with tritiated norepinephrine (10 ng/ml, 3.3 nCi/ml). All values represent the mean (horizontal bar) ± SEM (vertical bar) from four rats. Key: (*), postcyclazocine values significantly different from control values (C) at* $p < 0.05$.

[4] Sterling Winthrop Research Institute, Rensselaer, N.Y.
[5] Tween 80, Atlas Powder Co., Wilmington, Del.
[6] New England Nuclear Corp., Boston, Mass.
[7] Sigma Chemical Co., St. Louis, Mo.
[8] Geigy Pharmaceuticals, Ardsley, N.Y.

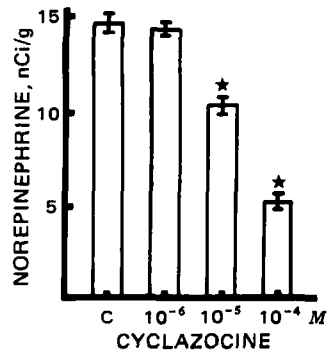

Figure 5—*The effect of various concentrations of cyclazocine on the uptake of tritiated norepinephrine by the isolated perfused rat heart. Hearts were removed and perfused with tritiated norepinephrine (10 ng/ml, 3.3 nCi/ml) in the absence (control C) and presence of 10^{-6}, 10^{-5}, or 10^{-4} M cyclazocine ± SEM (vertical bar) from four rats. Key: (*), cyclazocine-treated values significantly different from control values (C) at $p < 0.05$.*

into sympathetic nerves. Such an inference is supported by a significant cyclazocine-induced decrease in the formation of [^{3}H]dihydroxymandelic acid. Since monoamine oxidase appears to be principally located in the adrenergic neuron and has been shown to catalyze the oxidative deamination of intraneuronal norepinephrine, a decrease in neural uptake would be expected to result in a decrease in the deaminated metabolite of norepinephrine, dihydroxymandelic acid. In comparing the effect of desipramine, a compound that has been reported to decrease neuronal uptake in the rat heart (24), cyclazocine was less active in suppressing the accumulation of tritiated norepinephrine by the rat heart and did not produce a significant decrease in the formation of [^{3}H]normetanephrine (Figs. 1 and 3).

The observed changes in the recovery of radiolabeled normetanephrine from the heart induced by cyclazocine and desipramine were of interest in light of the reported increases in normetanephrine accumulation produced by agents that inhibit neuronal uptake in brain tissue (25, 26). These results are consistent with those presented previously (27, 28). It was shown that agents that inhibit adrenergic neuronal uptake (cocaine or imipramine) reduce the recovery of tritiated normetanephrine from heart tissue when [^{3}H]norepinephrine is administered intravenously. One explanation for the difference in the recovery of normetanephrine in brain and heart tissue produced by an agent that impedes neural uptake might be that the diffusion rate of the *O*-methylated metabolite out of the tissues varies markedly (29).

Since the *in vivo* results suggested a cyclazocine-induced inhibition of neuronal uptake, the effect of cyclazocine on the uptake of [^{3}H]norepinephrine was examined in isolated rat heart perfused with a concentration of norepinephrine (10 ng/ml) that when recovered would be indicative of neuronal uptake (19, 26). Cyclazocine added directly to the medium or given to the animal significantly decreased the uptake of tritiated norepinephrine into the isolated perfused heart, presumably at the level of the sympathetic nerve terminals.

Cyclazocine caused a depletion of endogenous cardiac norepinephrine at 10, 20, and 40 mg/kg. Cyclazocine has been shown to decrease levels of norepinephrine in brain tissue (30). It could be suggested that the depletion of endogenous cardiac norepinephrine was due to an inhibition of uptake; however, such a conclusion is not warranted, since tricyclic antidepressants with potent neuronal uptake inhibitory actions do not deplete catecholamine stores (31, 32). One possible mechanism is that cyclazocine releases norepinephrine, thereby, reducing stores of the catechol in the rat heart.

An earlier study (33) can be cited in support of such an alternate hypothesis. It was theorized that pentazocine, a chemically related compound to cyclazocine, produces a calcium-dependent action on vasculature and that the ability of pentazocine to decrease norepinephrine concentrations is related to an enhanced calcium influx into the nerve terminal, causing a release and subsequent depletion of the neurotransmitter.

Although the results presented in this paper suggest that cyclazocine interacts with the sympathetic nervous system by impeding neuronal uptake, they do not negate the possibility that cyclazocine alters sympathetic neural activity by other actions, *e.g.*, by enhancing release of neurotransmitter.

Furthermore, the results add support to the hypothesis that cyclazocine can induce central nervous system effects by not only interacting with opiate receptors but by altering adrenergic nerve function (34, 35).

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ACKNOWLEDGMENTS

Supported in part by grants from the University of Kansas and The Kansas Heart Association.