two separate and distinct reactions are necessary for C'3 lysis. The first is a combination of C'3NeF found in nephritic pseudoglobulin (Table 1) with a cofactor in normal pseudoglobulin which occurs rapidly and is dependent on the presence of Mg^{++}. Separately, neither the C'3NeF nor the cofactor exert any discernible effect on the C'3 molecule; their product, however, causes lysis of C'3. This combination of C'3NeF and its cofactor, therefore, will be referred to as the C'3 lytic nephritic factor (C'3 LyNeF). These reactions are summarized below:

$$\text{C'3NeF} + \text{cofactor} \xrightarrow{Mg^{++}} \text{C'3LyNeF} \quad (1)$$

$$\text{C'3LyNeF} + \text{C'3} \longrightarrow \beta_{1A} + \alpha_{2D} \quad (2)$$

A similar reaction sequence has recently been described by Müller-Eberhard as occurring between a factor in cobra venom and a β-globulin normally present in human serum (*7*). In the presence of bivalent cations, this reaction yields a C'3 inactivator complex which converts C'3 into C'3i. As with the active principles in the cobra venom reaction, neither the nephritic factor nor its cofactor are identifiable as components or products of the complement system. It has not been possible to identify C'3NeF as convertase enzyme (C'4,2a complexes) (*8*) for the reasons that (i) convertase present in nephritic serum would not require a cofactor for its activation and (ii) differing from convertase, C'3NeF is extremely stable, retaining its activity after incubation of nephritic serum for 7 days at 37°C.

There is also evidence that C'3NeF is not aggregated gamma globulin, antigen-antibody complexes, or antibody-complement complexes of the form AbC'1a,4. To ascribe C'3NeF activity to these complexes, the assumption must be made that some or all of the first three reacting components of complement are absent from the nephritic serum. In this event, the addition of normal pseudoglobulin to the nephritic serum would supply the missing component or components and a bivalent cation-dependent reaction would occur to form convertase. The convertase, like C'3LyNeF, would then break down C'3 in the absence of bivalent cations. Such an assumption, however, seems untenable. Although C'1, C'2, and C'4 have not been directly measured in the specific nephritic serum in these studies, these components have previously been shown (*9*, *10*) to be present in adequate concentrations in the serums of other patients with hypocomplementemic persistent nephritis. Further evidence against identification of C'3LyNeF with convertase is provided by the observations that (i) treatment of normal pseudoglobulin with iodine to form oxy-C'2 (*11*) before addition of nephritic pseudoglobulin caused no enhancement of the rate of the breakdown of C'3 and (ii) whereas the half-life of convertase is only 12 minutes at 32°C (*8*), C'3LyNeF retained full activity when incubated for 40 minutes at 37°C as a mixture of nephritic and normal pseudoglobulin. The C'3LyNeF also differs from the C'3 inactivator described by Tamura and Nelson (*3*) in the C'3LyNeF causes cleavage of fluid-phase C'3.

The role of this C'3 lytic system in the pathogenesis of either the reduced β_{1C} levels or the glomerulonephritis itself is not known at present. Conceivably, this system, if present in the glomerulus, could initiate the renal inflammation by activating the last six complement components. Requirements for an immune complex and convertase enzyme would, therefore, be bypassed. On the other hand, C'3NeF may be part of a protective mechanism; the destruction of serum C'3 would reduce the amount of this component deposited on the glomerulus, thus partially preventing activation of subsequent components. The elucidation of the precise function of this system may be an important step in understanding the biological role of complement in glomerulonephritis.

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12. Supported in part by a PHS fellowship 5 FO3 AM36243-02 from the National Institute of Arthritis and Metabolic Diseases (R.E.S.) and by a fellowship from the National Kidney Foundation (E.H.V.).

16 December 1968

Tricyclic Antidepressants: Evidence for an Intraneuronal Site of Action

Abstract. *Desipramine, a tricyclic antidepressant drug, almost completely prevents the accumulation of tritiated norepinephrine by sympathetic neurons of the rat heart after the injection of a tracer dose of the labeled amine. However, desipramine does not alter the accumulation of norepinephrine after the injection of a large dose of the neurohormone. Despite the failure of desipramine to block the neuronal uptake of norepinephrine, it still prevents exogenous norepinephrine from displacing the endogenous neurohormone (previously labeled with H^3-norepinephrine) from intraneuronal storage sites.*

Biochemical and histochemical evidence indicates that adrenergic neurons concentrate norepinephrine (NE) by two mechanisms; one, at the neuronal membrane, takes up NE from the circulation and from sympathetic receptors; another within the neuron incorporates the amine into storage vesicles (*1*). The ability to inhibit the uptake mechanism in the neuronal membrane is a characteristic of tricyclic drugs, such as imipramine, desipramine, and amitriptyline, which are widely used agents in therapy of mental depression (*2*). Since this mechanism is mainly responsible for the termination of the effects of NE, the tricyclic drugs are believed to act by prolonging the association of the neurohormone with peripheral receptor sites after nerve stim-

Table 1. Effect of desipramine (20 mg/kg, intraperitoneally, given 1 hour before administration of *l*-NE) on displacement of H^3-NE from rat heart by *l*-NE (2 mg/kg, intravenously). Abbreviation: dpm, disintegrations per minute.

Time after *l*-NE (min)	Treatment	Number of animals	NE (μg/g ± S.E.)	H^3-NE (10^3 dpm/g ± S.E.)	Displacement of H^3-NE (%)
0	None	6	1.11 ± .03	193 ± 23	
0	Desipramine	6	1.00 ± .03	220 ± 24	
20	None	7	2.46 ± .14	130 ± 12	33
20	Desipramine	7	2.28 ± .31	174 ± 19	21
180	None	7	1.32 ± .05	82 ± 8	58
180	Desipramine	7	1.17 ± .07	176 ± 23	20

ulation (*3*). The antidepressant action of desipramine is generally attributed to similar effects in the brain (*4*).

Recent studies, however, raise the possibility that desipramine may act on the concentrating mechanism of storage vesicles as well as on the neuronal membrane. Schildkraut *et al.* observed that imipramine decreases the urinary excretion of deaminated metabolites of NE in patients and suggested that an intraneuronal action of the antidepressant might hinder the NE in storage vesicles from diffusing onto mitochondrial monoamine oxidase (*5*). Further evidence that desipramine acts intraneuronally is the demonstration that the drug decreases the rate at which reserpine releases NE from heart and brain (*6*). Since reserpine releases NE through an action on storage vesicles (*7*), these results suggest that desipramine has an action on storage vesicles that might be associated with the prevention and reversal of reserpine-induced sedation in rats and man (*8*).

The tricyclic antidepressants can inhibit the neuronal uptake not only of NE but also of other amines, including metaraminol, guanethidine, and tyramine (*9*). Desipramine also interferes with the release of NE by these amines, and this action is generally attributed to blocking of the amine transport mechanism in the neuronal membrane (*10*). However, recent reports suggest that desipramine interferes with the release of NE by more than one mechanism. For example, Leitz and Bogdanski have concluded that desipramine interferes with the metaraminol-induced release of NE from rat heart slices, in part by reducing the uptake of metaraminol into nerve endings, and in part by interfering with the releasing action of metaraminol within the neuron (*11*). Similar conclusions have been drawn from studies with metaraminol and tyramine in vivo (*12*). Finally, desipramine blocks the depletion of heart NE by amphetamine (*13*), a finding which strongly suggests an intraneuronal action since desipramine and other inhibitors of membrane transport do not affect the accumulation of liposoluble amphetamine in tissues (*14*). To show that desipramine may act partly by preventing these amines from gaining access to the storage vesicles, we present evidence that desipramine prevents exogenous NE taken up by sympathetic neurons from freely mixing with endogenous stores of the neurohormone.

We first administered a tracer amount (*15*) of H^3-norepinephrine (H^3-NE) (0.165 μg/kg, intravenous injection) to male Sprague-Dawley rats (190 g). We killed the animals at various times thereafter and assayed NE and H^3-NE in heart as described (*16*). H^3-Norepinephrine was readily taken up by the heart of control animals (Fig. 1). The slow disappearance of H^3-NE at a rate corresponding to the turnover rate of the endogenous amine indicated that the intraneuronal stores of neurohormone had been labeled (*17*). In contrast, after treatment with desipramine the incorporation of H^3-NE was largely blocked, and the small amounts of labeled NE taken up by the heart disappeared at a relatively rapid rate.

We next administered a large dose of *l*-NE (2 mg/kg, intravenous injection) to rats (*18*). Within 5 minutes the NE stores in heart of control rats increased about fivefold, then declined to 2.3 μg/g during the next 15 minutes and thereafter gradually decreased to the normal value (Fig. 2). Administration of desipramine did not reduce significantly the amount of *l*-NE taken up by the heart nor did it affect the time course of its disappearance. In contrast, when intraneuronal storage of NE was blocked by a maximally effective dose of reserpine (*19*), the heart failed to retain exogenous *l*-NE (Fig. 2). Comparing the accumulation of NE in reserpine-treated rats to that in control and desipramine-treated animals, we infer that after administration of desipramine the exogenous amine was probably incorporated into sympathetic neurons. Thus, in contrast to the desipramine-induced blockade of H^3-NE uptake after injection of a tracer dose of the amine, the antidepressant agent does not prevent the neuronal accumulation of NE after administration of a large dose of the neurohormone.

A possible intraneuronal action of desipramine was revealed by experi-

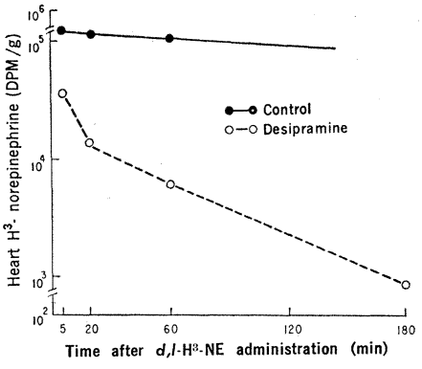

Fig. 1. Disappearance of H^3-NE taken up by heart after administration of a tracer dose of *d,l*-H^3-NE (0.165 μg/kg, intravenous injection) to control rats and to rats treated with desipramine (20 mg/kg, intraperitoneally) 1 hour previously. Each point represents a mean value of at least five animals.

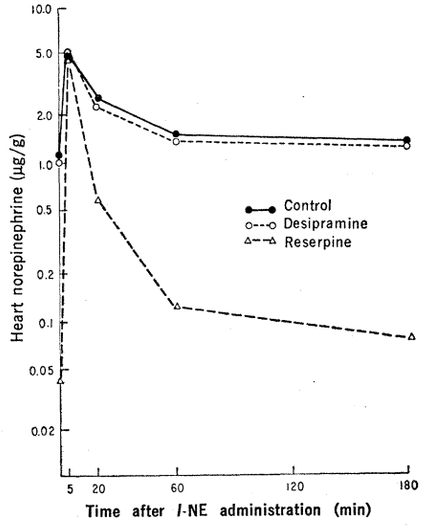

Fig. 2. Amounts of NE in heart after administration of *l*-NE (2 mg/kg, intravenous injection) to control rats and to rats treated with desipramine (20 mg/kg, intraperitoneally) 1 hour previously or with reserpine (5 mg/kg, intravenous injection) 4 hours previously. Each point represents a mean value of at least five animals.

ments in which the drug apparently inhibited the movement of exogenous NE into storage sites. In control rats whose cardiac NE stores were previously labeled by H^3-NE (50 μc/kg, intravenous injection, 24 hours previously), the intravenous injection of *l*-NE (2 mg/kg) reduced the heart H^3-NE by 60 percent (Table 1; typical of three experiments). In rats previously treated with desipramine, the *l*-NE displaced only 20 percent of the H^3-NE, though, as shown above, the neuronal accumulation of *l*-NE was virtually unchanged. After a larger dose of *l*-NE (10 mg/kg, intramuscularly) about 90 percent of heart H^3-NE was displaced in control rats compared to 45 percent in animals treated with desipramine (*20*). These results indicate that the exogenous *l*-NE taken up by adrenergic neurons displaced a considerable fraction of the endogenous amine from storage sites; previous treatment with desipramine appears to have interfered with this displacement.

In conclusion, our results add to accumulating evidence that desipramine, and perhaps other tricyclic antidepressants, exert an intraneuronal action. Admittedly, the action of desipramine at the neuronal membrane can account for the blockade of NE uptake after administration of tracer doses of the catecholamine. However, when a large dose of *l*-NE is injected, desipramine does not block neuronal uptake, yet it apparently prevents the exogenous amine from freely mixing with endogenous NE stores labeled with H^3-NE. Since the storage vesicles contain most of the endogenous neurohormone (*21*), we favor the view that desipramine may act also on storage vesicles to prevent *l*-NE and other amines from displacing endogenous NE. Whether or not this intraneuronal action of desipramine bears any relationship to the pharmacological mechanism by which the drug produces its characteristic clinical effects remains unanswered.

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18. Rats were protected against the lethal sympathomimetic effects of NE by the injection of phenoxybenzamine (3 mg/kg, intraperitoneally) 3 hours previously.
19. A maximally effective dose of reserpine has been defined as one which depletes heart NE at a maximal rate constant [W. D. Reid and B. B. Brodie, in *Mind as a Tissue*, C. Rupp, Ed. (Hoeber Medical Division, Harper and Row, New York, 1968), p. 87].
20. Experiments with this dose of *l*-NE (10 mg/kg, intramuscularly) were performed by N. H. Neff, W. H. Hammer, and T. N. Tozer, and confirmed by us.
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21 November 1968; revised 11 February 1969

Resistance to Metronidazole by Trichomonas foetus in Hamsters Infected Intravaginally

Abstract. *Induced resistance to the antitrichomonad agent metronidazole administered to hamsters infected intravaginally with* Trichomonas foetus *and treated with subcurative levels of metronidazole was demonstrated in vitro and in vivo.*

Metronidazole [1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole] is effective against trichomoniasis, but it has been suggested that resistance to it could become clinically significant (*1*). Several workers have shown that resistance to metronidazole by *Trichomonas vaginalis* can be induced in vitro (*2*). There is apparently only one reference to the characteristics in vivo of these resistant organisms (*3*). We have no report on the induction of resistance in vivo.

Hamsters (*Mesocricetus auratus*, young virgin females) were infected intravaginally with *Trichomonas foetus* and subsequently treated orally with suboptimum doses of metronidazole (50 mg/kg; 12 doses over a 3-week period). The trichomonas organism was then isolated and compared with the parent strain for sensitivity to metronidazole. The assay system employed was a twofold tube dilution assay in Diamond's medium (10 per-

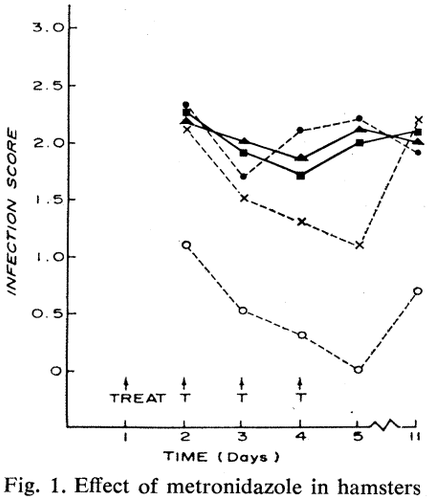

Fig. 1. Effect of metronidazole in hamsters infected intravaginally with a metronidazole-sensitive or a metronidazole-resistant strain of *Trichomonas foetus*. ▲—— Infection control, resistant strain; ■—— metronidazole (200 mg/kg), resistant strain; ● - - - - infection control, sensitive strain; × - - - - metronidazole (50 mg/kg), sensitive strain; and ○ - - - - metronidazole (100 mg/kg), sensitive strain.