Release of Metaraminol (Aramine) from the Heart by Sympathetic Nerve Stimulation

Abstract. *Cats were injected intravenously with metaraminol, an amine which depletes norepinephrine from the sympathetic nerve endings; their hearts were then perfused in vitro. Stimulation of the sympathetic nerve supply released metaraminol from the hearts, demonstrating that a synthetic compound can replace norepinephrine and serve as a transmitter at adrenergic nerve endings.*

Metaraminol (Aramine) is a sympathomimetic amine widely used in clinical medicine as a vasopressor agent. In addition to stimulating adrenergic receptors, metaraminol also depletes norepinephrine from the peripheral sympathetic nerve endings (*1–4*). In recent studies of the mechanism of this norepinephrine-depleting action, Udenfriend (*1*) and Gessa *et al.* (*2*) have reported that the amount of norepinephrine lost from the heart greatly exceeds the amount of metaraminol taken up by the heart (in guinea pigs and rats), indicating that depletion of the normal transmitter cannot be attributed to a mole-for-mole displacement of norepinephrine by metaraminol. Carlsson and Lindqvist (*3*), on the other hand, have suggested that a stoichiometric exchange of these amines can account for their data obtained in experiments with the mouse brain. Recent studies in this laboratory lend support to this latter view (*5*). It was found, for example, that metaraminol is rapidly taken up from the circulation by the rat heart, that the degree of uptake parallels the degree of norepinephrine loss, and that reserpine can release this bound metaraminol from the heart—all of which suggest that metaraminol can substitute directly for norepinephrine in the sympathetic nerve endings.

If it is true that metaraminol is bound at sites ordinarily occupied by norepinephrine, one would expect sympathetic nerve stimulation to release metaraminol from the hearts of animals previously treated with metaraminol, just as nerve stimulation releases norepinephrine from the hearts of normal animals. The experiments described here were designed to test this possibility.

Adult female cats weighing 2.2 to 4.0 kg were anesthetized with sodium pentobarbital and given a single intravenous injection of *l*-metaraminol, 100 μg per kilogram of body weight. Either 2 hours later or 17 to 20 hours later, the heart was removed with the adjacent mediastinum, the right stellate ganglion, and a portion of the right sympathetic chain intact. A glass cannula was quickly tied into the aorta, and the heart was perfused at 37°C by the Langendorff technique with Krebs-Henseleit solution saturated with 95 percent O_2 and 5 percent CO_2; the perfusion pressure was 60 cm of water. (In this preparation the perfusion solution enters the coronary circulation from the aorta, returns to the right atrium by way of the coronary sinus, flows out of the openings where the great veins were cut off and down the surface of the heart into a collecting funnel.) Flow rates in these experiments ranged from 15 to 60 ml/min. The apex of the heart was attached to a Grass force transducer for the recording of rate and tension. Each heart was allowed to stabilize on the perfusion apparatus for 10 to 15 minutes. The right sympathetic chain was then stimulated at supramaximal voltage for a period of 10 seconds at 25 impulses per second (square-wave pulses, 1 msec duration). The effluent was collected during a 30-second control period and during the first two 30-second periods after the beginning of nerve stimulation. Five minutes later an injection of 0.3 μg *l*-norepinephrine was given by way of the perfusion cannula, the effluent again being collected as described. A second period of nerve stimulation and a second norepinephrine injection were then given at 5-minute intervals. In some experiments an additional norepinephrine injection was given prior to the first period of nerve stimulation. At the end of the experiment the left ventricular myocardium was assayed for norepinephrine (*6*) and for metaraminol (*7*). Metaraminol was extracted from the effluents with *n*-butanol after saturation of the aqueous phase with solid sodium chloride. The amine was returned from the butanol to an acid phase with the aid of 1.5 volumes of heptane for each volume of butanol, and was assayed fluorimetrically (*7*).

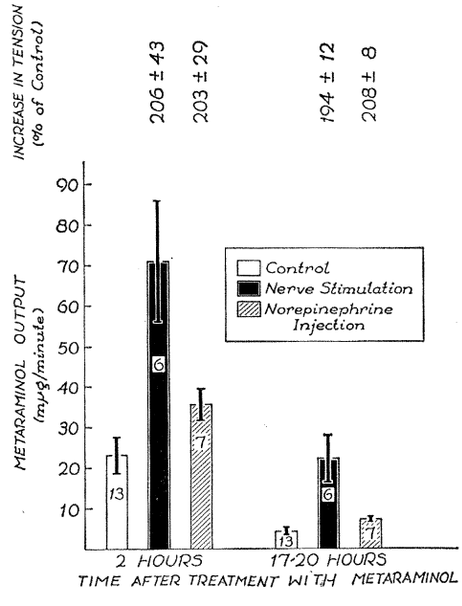

Fig. 1. Output of metaraminol from perfused cat heart with intact sympathetic innervation during control period, during first minute after sympathetic nerve stimulation, and during first minute after norepinephrine injection. Bars on left show mean ± standard error for 13 control periods, six periods of nerve stimulation and seven norepinephrine injections in four cats; bars on right show similar results in three cats. Numbers at top show that increase in tension was essentially the same in all groups.

Figure 1 shows the combined results of 12 periods of nerve stimulation and 14 injections of norepinephrine in seven hearts. Although both sympathetic nerve stimulation and norepinephrine released metaraminol, nerve stimulation released considerably more. Since the norepinephrine injections duplicated the hemodynamic effects of nerve stimulation (top of Fig. 1), the much larger release of metaraminol by nerve stimulation cannot be attributed simply to a mechanical "squeezing-out" of metaraminol from nonspecific sites by an increased force of contraction of the myocardium. In control experiments, it was found that direct electrical stimulation of the myocardium did not release metaraminol. Finally, nerve stimulation was performed on four occasions in two preparations while nethalide, a β-adrenergic blocking agent, was delivered to the heart at a rate of 50 μg/min. Under these conditions, nerve stimulation produced no change in cardiac rate or tension but still released metaraminol.

The contribution of metaraminol to the increase in rate and contractile force produced by nerve stimulation cannot be assessed from these studies,

since assays of the ventricular myocardium at the end of each experiment revealed that considerable amounts of norepinephrine were still present in the nerve endings. The four hearts studied 2 hours after the injection of metaraminol contained a mean concentration of 0.55 μg of metaraminol per gram of tissue (wet weight) and 0.80 μg/g of norepinephrine. In the three hearts studied at 17 to 20 hours, these values were 0.46 μg/g and 0.31 μg/g, respectively. It is interesting that the amount of metaraminol released was much greater in hearts studied at the early time period (Fig. 1), although the concentration of metaraminol in the left ventricular myocardium was nearly the same in both groups. This suggests that metaraminol taken up by the heart can shift with time from an "available" to a "less readily available" pool, as suggested for norepinephrine (*8*).

In other studies it has been observed that tyramine, an amine which is known to release norepinephrine from adrenergic nerve endings (*9*), can also release metaraminol from the perfused cat heart. This provides additional evidence for the view that metaraminol is bound in the nerve endings at sites ordinarily occupied by norepinephrine. Muscholl and Maitre (*10*) have recently reported that α-methylnorepinephrine, a metabolite of α-methyl-dopa (*3*), can also be released from the heart by stimulation of the sympathetic nerves. Their study and this one therefore provide the first direct evidence that synthetic compounds can be released as neurohumoral transmitters from adrenergic endings. Since metaraminol is much less potent than the normal transmitter, norepinephrine, it is possible that more extensive replacement of norepinephrine by metaraminol than was achieved in these experiments could produce a form of adrenergic nervous system blockade. It is commonly observed that patients given large doses of metaraminol by infusion are hypotensive for varying periods of time after the infusion; perhaps a "transmitter substitution" mechanism plays a role in the pathogenesis of this type of hypotension.

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Thysanuran Median Frontal Organ: Its Structural Resemblance to Photoreceptors

Abstract. *The median frontal organ of* Thermobia domestica *(Packard) and the median ocellus of* Tricholepidion gertschi *Wygodzinsky were compared with respect to location, innervation, and fine structure. The data suggest that the two organs are homologous. Elementary neurosecretory granules were not found in the median frontal organ, but multivesicular bodies are present in both the frontal organ and ocellus.*

Because the Thysanura (Bristle-tails) seem more closely related to pterygote insects than do any of the other extant apterygote orders (*1*) there is a distinct possibility that clarification of hormonal mechanisms operative in this group may provide clues toward an interpretation of comparative arthropod endocrinology. Experimental studies on thysanuran endocrine systems were commenced only recently (*2*) and emphasize the need for detailed anatomical information.

The cephalic endocrine system in this group of insects was reviewed by Watson (*3*). In addition to the paired lateral frontal organs which consist of components with structural and tinctorial characteristics of neurosecretory cells, an unpaired median frontal organ has been described (*4–8*). The latter structure seems to be absent in members of the family Machilidae, but present in many Lepismatidae (*4, 6*). Since it has been postulated that the median frontal organ may serve an endocrine function (*5*), we decided to compare the fine-structural features of this organ in *Thermobia domestica* (Packard) with those of known neurosecretory cells (*9*).

In *Thermobia*, the median frontal organ (Fig. 1) lies just beneath the anteromedial region of the frons, above the epistomal suture, and between the frontostomodaeal muscles (*8*). We were able to distinguish the location of this subellipsoidal organ by external examination of living specimens with a dissecting microscope, for it is light tan and visible through the transparent frontal cuticle after removal of the scales. On the basis of its gross anatomy as seen in frontal section, one could subdivide the median frontal organ of *Thermobia* into a medial body and two transverse processes (*5*), but there was no cytological distinction between the cells of these subdivisions as far as we could determine. Histological examination revealed an unpaired nerve which leaves the organ, passes medioposterior-

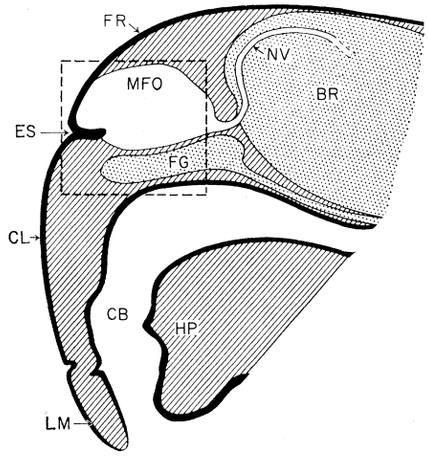

Fig. 1. Diagram of a sagittal section through the head of *Thermobia*. An area comparable to that shown in Fig. 2*A* is indicated by broken lines. (*BR*, brain; *CB*, cibarium; *CL*, clypeus; *ES*, epistomal suture; *FG*, frontal ganglion; *FR*, frons; *HP*, hypopharynx; *LM*, labrum; *MFO*, median frontal organ; *NV*, protocerebral nerve of *MFO*).