Uptake of Norepinephrine as a Determinant of the Magnitude of the Inotropic Response* (33534)

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The uptake of norepinephrine (NE) by sympathetic nerve endings has been proposed as an important mechanism for the inactivation of the circulating amine (1–8). If this is true the capacity of the nerve endings to take up NE should be an important factor in the determination of the magnitude of the responses of a sympathetically innervated structure to the circulating catecholamine. Thus, the nerve ending and the adrenergic receptor might be considered to compete for the circulating NE. Since isolated perfused hearts normally vary in their responses to NE and in the amounts of NE that they are able to take up a study of the relation between these two parameters appeared to be a possible way to assess the validity of this hypothesis. Also, since it might be argued that uptake of NE by other than nerve terminals could account for any variability in uptake observed, experiments utilizing cocaine to block neuronal uptake (9) were performed. Finally, the relationship of response to uptake of isoproterenol, a substance taken up at the concentration employed to only a limited extent by nerve endings (10), was determined.

Materials and Methods. Male albino rats of the Holtzman strain weighing 250–300 g were given heparin sodium (500 U/kg) intraperitoneally. Ten min later the animals were killed by decapitation, the hearts were removed and perfused by the Langendorff technique. The perfusing fluid was a warm (37°C) oxygenated (95% O_2, 5% CO_2) Nasmyth solution having the following composition in g/liter: NaCl, 6.900; KCl, 0.350; $CaCl_2$, 0.280; $MgSO_4$, 0.293; NaH_2PO_4, 0.162; $NaHCO_3$, 2.100; glucose, 2.000. The rate of perfusion was maintained constant at 9 ml/min. The tip of a heart lever (Harvard Apparatus Co.) was inserted as near the ventricular apex as possible in order to transmit the contractions to a transducer and display them in a Sanborn 964 recording system. After perfusing the heart for 5 min in order to wash out residual blood 5 measurements of rate and contractile force were obtained at intervals of 1 min. A solution containing 0.012 μg of NE-^{3}H [(±)-norepinephrine-7-^{3}H hydrochloride, 10.1 Ci/mmole, New England Nuclear Corp.] and 5 μg of (±)-NE hydrochloride in 0.5 ml was then injected directly into the perfusion canula over a period of 15 sec. Contractile force and rate were measured for 2 min, beginning with administration of the NE. Five min later the hearts were removed, weighed, and homogenized in 10 ml of cold 0.4 *N* $HClO_4$. The supernatant fluid was used for estimation of NE-^{3}H by the method of Axelrod *et al.* (8). The NE-^{3}H was eluted from the aluminum oxide columns with 10 ml of 0.2 *N* HCl. A 2-ml aliquot of the eluate was added to 10 ml of 1,4-dioxane and radioactivity was estimated in a Packard Tricarb liquid scintillation counter (model 3214, Packard Instrument Company Inc.). Counting efficiency was determined by automatic external standardization. Normetanephrine and deaminated catechols were determined by the method of Kopin *et al.* (11).

In a second series of 25 experiments 5 μg of isoproterenol hydrochloride and 0.021 μg of isoproterenol-^{3}H hydrochloride (5.03 Ci/mmole, New England Nuclear Corp.) were utilized instead of NE-^{3}H. Isoproterenol-^{3}H was estimated by dissolving the entire heart in 5 ml of NCS reagent (Nuclear Chicago) and determining the activity of a 2-ml aliquot in 15 ml of toluene. In still another series of 20 experiments the hearts were perfused with

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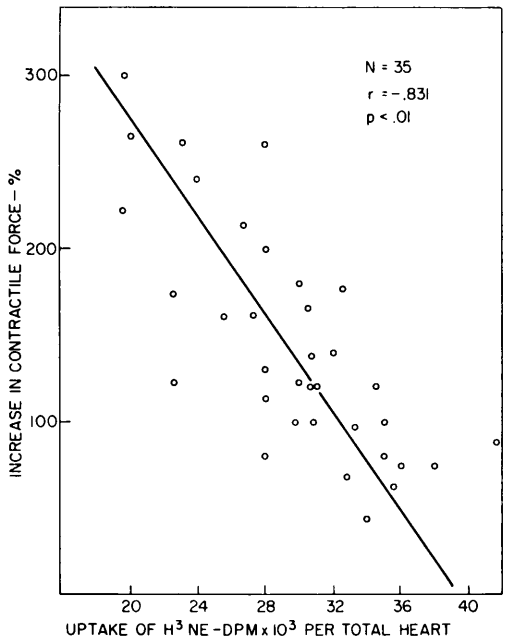

FIG. 1. Relation between the magnitude of the inotropic response to norepinephrine (percentage of control) and the uptake of its tritiated isomer (dpm $\times 10^3$ per heart) in isolated perfused rat hearts.

cocaine hydrochloride (10^{-5} *M*) for 15 min prior to the injection of NE-^{3}H.

Results and Discussion. Essentially all (98%) of the radioactivity in the hearts was unchanged NE-^{3}H. One % was normetanephrine and 1% was deaminated catechols. Figure 1 illustrates the relationship between increase in contractile force (as a percentage of control) and the amount of NE-^{3}H taken up by the heart after administration of NE-^{3}H. Uptake of the amine is inversely related to the inotropic response, i.e., the greater the uptake the smaller the response, and vice versa. Similar results ($r = -0.718$) were obtained when the uptake was expressed as dpm (disintegrations per minute)/mg of heart. No significant correlation was obtained when the uptake of NE-^{3}H was plotted against the chronotropic response.

The variability of the inotropic responses of the 25 hearts exposed to isoproterenol was significantly less ($F = 3.32$, $p < .01$) than the variability of responses to NE. The uptake of isoproterenol-^{3}H was much less than was that of NE-^{3}H and there was no correlation between the former and the magnitude of the inotropic response. Similarly, uptake of NE-^{3}H after treatment of hearts with cocaine was markedly reduced, was not correlated with the inotropic response and the variability of the response was significantly less ($F = 2.83$, $p < .01$).

The concept that the sympathetic nerve endings can take up and store circulating NE, thereby functionally inactivating it and terminating its pharmacological action, is supported by the results of many investigators. However, the relation between this uptake and the magnitude of the inotropic and chronotropic responses has not been evaluated. The results presented here support the hypothesis which envisions the uptake of NE by sympathetic nerve endings as an important mechanism in the determination of the extent of the adrenergic response because (i) an inverse correlation between uptake of NE-^{3}H and degree of inotropic response could be demonstrated, (ii) pretreatment of hearts with cocaine reduced the variability of the responses of NE-^{3}H, the uptake of the amine, and abolished the correlation between uptake and response, and (iii) uptake, variability of response and correlation of response with uptake when isoproterenol (a drug poorly taken up by nerve endings) was employed instead of NE-^{3}H were similar to the findings with NE-^{3}H administration after cocaine.

Failure to obtain such a relationship when the chronotropic response was plotted against the uptake of NE-^{3}H is not too surprising, since cardiac rate at any given time will be determined by the cell or group of cells discharging impulses at the highest rate, i.e., in the sinus mode. To determine whether uptake of NE is in any way related to chronotropic responses one therefore would have to seek the answer in relation to that cell or those few cells which are responsible for the response. On the other hand, the inotropic response is the sum of the responses of all the contractile fibers in the heart, hence a relationship of this response to uptake of NE by sympathetic nerve terminals in the entire heart would be expected.

Summary. The magnitude of the inotropic and chronotropic responses to norepinephrine and uptake of its tritiated isomer (NE-^{3}H) were studied in isolated perfused rat hearts. A statistically significant inverse correlation between the uptake of (NE-^{3}H) and the magnitude of the inotropic response was demonstrated. This correlation disappeared when the hearts were pretreated with cocaine or when uptake of and response to tritiated isoproterenol rather than NE-^{3}H were examined. The variability of the inotropic responses to norepinephrine after cocaine or to isoproterenol were significantly less than responses to norepinephrine in the absence of any pretreatment. These results support the hypothesis which envisions the neuronal uptake of norepinephrine as an important mechanism in the determination of the magnitude of the adrenergic response to exogenous norepinephrine.

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