Sodium Dependence of Transmitter Uptake at Adrenergic Nerve Terminals

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SUMMARY

The uptake of norepinephrine into the sympathetic innervation of the isolated rat heart was dependent on the presence of sodium ions in the external medium, but was not affected by the absence of calcium ions. The sodium dependence of norepinephrine uptake is further evidence for the view that this process is mediated by an active transport system in adrenergic nerve terminals. Similar sodium-dependent uptake processes may be present at other chemically transmitting synapses.

Tissues with a sympathetic innervation, such as the heart, rapidly take up exogenous norepinephrine (1, 2). This uptake is severely reduced when the sympathetic postganglionic innervation is destroyed by surgical denervation or by immunosympathectomy, suggesting that the sites of uptake are located in the sympathetic nerve terminals (for reviews see 3, 4). Kinetic studies of norepinephrine uptake in the isolated rat heart have indicated that the uptake is mediated by an active transport system, presumably located in the membranes of the sympathetic nerve terminals (1). It has been suggested that the physiological effects of norepinephrine are terminated by a rapid uptake of the released transmitter back into the presynaptic nerve terminals at adrenergic synapses; thus the uptake process may play a role analogous to that of acetylcholinesterase at cholinergic synapses (3–5).

In the present study we have found that the uptake of norepinephrine into the sympathetic innervation of the rat heart is dependent on the presence of sodium ions in the external medium; since many other active transport processes are known to share this property (6, 7), this finding represents further evidence for the existence of an active transport process for norepinephrine in adrenergic nerves.

Hearts from adult male Sprague-Dawley rats were perfused as previously described (1). A preliminary perfusion for 5 min with the test medium was followed by a 10-min perfusion with the test medium containing DL-7-^{3}H-norepinephrine (New England Nuclear Corp., Boston, Massachusetts; specific activity = 7.12 C/mmole; electrophoretically pure) at a concentration of 1.1 mμg/ml. The accumulation of ^{3}H-norepinephrine in the tissue was determined as previously described (1). Normal medium was a modified Krebs-Henseleit solution at 37°, gassed with a mixture of 95% oxygen and 5% carbon dioxide. Sodium-deficient media, containing 17% and 44% of the normal sodium concentration, were prepared by replacing a part of the sodium

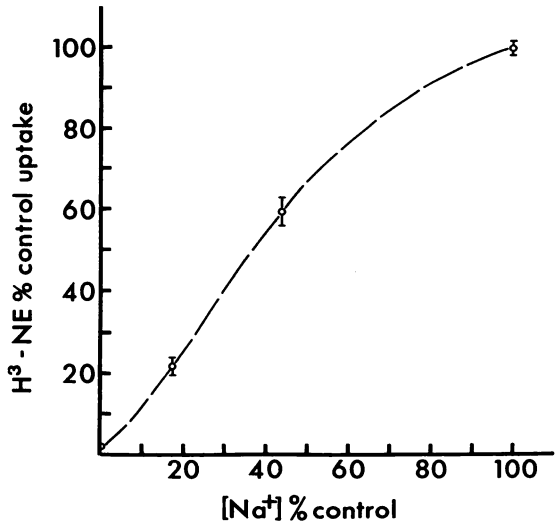

FIG. 1. *The uptake of ³H-norepinephrine in the isolated rat heart perfused with sodium-deficient media containing* DL-*³H-norepinephrine (1.1 mμg/ml)*

Values are expressed as percentages of sodium concentration and ³H-norepinephrine uptake in control hearts and are means ± S.E. Mean for groups of 6 hearts. Sodium concentration of control medium was 145 mM; mean uptake of ³H-norepinephrine in control hearts was 20.3 mμg/g wet weight/10 min.

chloride with isotonic sucrose (0.3 M). A "sodium-free" medium was prepared by completely replacing sodium salts with the corresponding lithium salts.

Hearts perfused with the "sodium-free" medium failed to beat, and the mean perfusion rate was reduced to 4.9 ml/min, compared with a mean rate of 10.2 ml/min in control hearts. The rate of norepinephrine uptake is to some extent dependent on the rate of perfusion of the tissue. However, reducing the perfusion rate with normal medium from 10–12 ml/min to 4–5 ml/min reduces the rate of norepinephrine uptake by less than 20% (Iversen, unpublished results). The reduced perfusion rate of the "sodium-free" group in the present experiments is clearly insufficient to account for the striking reduction in ³H-norepinephrine uptake in these tissues (Fig. 1). Furthermore, ³H-norepinephrine uptake was also considerably reduced in hearts perfused with the other sodium-deficient media (Fig. 1), even though in these two groups the perfusion rates were not significantly different from that in controls. Hearts perfused with 17% sodium beat sporadically whereas those perfused with 44% sodium beat regularly.

In similar experiments it was found that the rate of uptake of norepinephrine in this preparation was not significantly altered by changing the calcium concentration of the perfusing medium from zero to twice the normal value. As in "sodium-free" media, hearts did not beat when perfused with calcium-free solutions.

These results show that the rate of uptake of norepinephrine into the sympathetic innervation of the rat heart is dependent on the presence of sodium ions in the external medium; lithium is almost totally ineffective as a substitute for sodium. Previous studies of the kinetics of norepinephrine uptake have suggested the existence of a carrier-mediated membrane transport process (1, 8). The finding that norepinephrine uptake is a sodium dependent process further supports this hypothesis, since many other active transport processes also require sodium (6, 7, 9, 10).

The presence of a sodium-dependent active transport system for the uptake of neurotransmitters or related substances into presynaptic nerve terminals may not be unique to adrenergic synapses. Other studies have suggested the existence of a choline uptake process in the presynaptic terminals

of cholinergic nerves. If the uptake of choline is blocked by drugs, or choline is omitted from the medium, cholinergic transmission is rapidly impaired owing to a decreased synthesis of acetylcholine and an exhaustion of the available transmitter stores (11). Omitting sodium from a choline-containing medium produces an identical effect, suggesting that the uptake of choline is also sodium dependent (12).

Similar active transport systems may exist at other chemically transmitting synapses. There is a strong possibility that γ-aminobutyric acid (GABA) and glutamic acid are transmitter substances at inhibitory and excitatory neuromuscular synapses, respectively, in crustacean muscle (13–15). We have recently found that a lobster nerve-muscle preparation can accumulate exogenous ^{3}H-GABA or ^{3}H-glutamic acid by two separate uptake mechanisms. Both these processes transport the amino acids into the tissue against a concentration gradient, and both uptake systems are sodium dependent (16; and unpublished observations).

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