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pH Dependence of [^{3}H]norepinephrine uptake into catecholamine storage vesicles isolated from rat brain, heart and adrenal medulla

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Synaptic storage vesicles play several important roles in catecholamine neurons and are directly involved in neurotransmitter synthesis, storage and release. Norepinephrine and its precursor, dopamine, are taken up and maintained within the vesicle by a transport system coupled to a Mg^{2+}-activated ATPase in the vesicle membrane [1–3]. Current research suggests that the coupling of the ATPase to amine transport is indirect, via a "chemiosmotic" mechanism involving proton-translocation and/or an electrical potential across the vesicle membrane [4–7]. One hypothesis proposes that the ATPase concentrates protons inside the vesicle, creating a pH gradient which then acts as the motive force for the subsequent steps of catecholamine transport. Thus, agents which render membranes permeable to protons inhibit catecholamine uptake into vesicles [2, 7, 8]. By indirect measurement, the interior of adrenomedullary storage vesicles (chromaffin granules) has been shown to have a pH of approximately 5.5 [9, 10], and in synaptic vesicle ghosts reconstituted from heart preparations reserpine-sensitive accumulation of norepinephrine has been demonstrated to require a pH gradient across the vesicle membrane [11].

Historically, most studies of vesicular uptake mechanisms have involved the chromaffin granules of the adrenal medulla, and it is generally assumed that synaptic storage vesicles from nerve terminals display similar properties [1–3]. Although neuronal preparations do display ion, nucleotide and substrate requirements similar to those of adrenomedullary vesicles [3, 12–14], some discrepancies have been noted [3, 5–9, 14]; however, because of the various preparative methods, tissues, and species involved in these comparisons, it is uncertain whether there are true differences among vesicle types. Accordingly, the current study examines in a single species (rat) the hypothesis that a similar proton-gradient-driven transport operates in vesicles from central nervous tissue (brain), peripheral nervous tissue (cardiac sympathetic nerves), and adrenal medulla.

Adult Sprague–Dawley rats (Zivic–Miller Laboratories, Allison Park, PA) were decapitated, and tissues were removed quickly and were homogenized (ground-glass apparatus for brain, Polytron for heart) in 4 vol. of ice-cold 300 mM sucrose buffered with 25 mM Tris–sulfate (pH 7.4) containing 10 μM iproniazid. A crude fraction containing synaptic vesicles was prepared by a modification of the methods of Seidler *et al.* [15] and Bareis and Slotkin [12]. Homogenates were centrifuged at 1,000 *g* for 20 min, the resulting supernatant fraction was centrifuged at 20,000 *g* for 45 min, and the second supernatant fraction was then sedimented at 100,000 *g* for 30 min. The crude vesicle pellet was then resuspended using three to four up–down strokes in a Teflon–glass homogenizer in 1.3 vol. (based on original wet weight tissue) of 130 mM potassium phosphate buffer at pH 7.4. The resuspensions from different animals were pooled, and aliquots were used for subsequent uptake determinations.

For determination of vesicular uptake, each sample contained 0.23 ml of vesicle suspension and 1.5 ml of 130 mM potassium phosphate buffered to obtain the various experimental pH values, along with final concentrations of 1 mM ATP-Mg^{2+}, 10 μM ascorbic acid, 5 μM iproniazid, and 0.1 μM *l*-[^{3}H]norepinephrine (4.5 Ci/mmole, New England Nuclear Corp., Boston, MA). Uptake blanks were determined in several samples at each pH by including 1 μM reserpine (Ciba Pharmaceuticals, Summit, NJ), a specific inhibitor of vesicular catecholamine uptake [16]. All samples were incubated for 4 min at 30°, and uptake was stopped by adding 2 ml of ice-cold phosphate buffer at the appropriate pH. The labeled vesicles were immediately vacuum-filtered and washed on cellulose acetate filter paper (Millipore type EG, pore size 0.2 μm). Filter papers were immersed in 10 ml of a Triton-containing scintillation mixture and counted at an efficiency of 40 per cent. Uptake was determined as the differences between samples without and with reserpine, and results are expressed as the mean ± S.E.M. of uptake as a percentage of the value at pH 7.4. Non-specific uptake (uptake in the presence of reserpine) was approximately 25 per cent of the total uptake at pH 7.4; disintegrations per minute in the reserpine-containing samples declined slightly vs pH.

For adrenomedullary vesicles, the original homogenate contained 2 glands/ml of 300 mM sucrose buffered with 25 mM Tris–sulfate (pH 7.4) and was centrifuged at 800 *g* for 10 min. Aliquots (0.062 ml) of the resulting supernatant fraction were then added to 0.44 ml of sucrose–Tris buffered to obtain the desired final experimental pH values; the resulting samples contained 5 mM ATP-Mg^{2+} and 0.3 μM *l*-[^{3}H]norepinephrine. Blank uptake values were obtained again with 1 μM reserpine. Samples were incubated for 15 min at 30° and vesicles were trapped and washed as described above. The preparative procedures and incubation conditions for adrenomedullary vesicles differ from those of synaptic vesicles due to the different sedimentation characteristics of the vesicles and different optimal conditions for time, substrate and cofactors; although each of these procedures uses a crude vesicle preparation, numerous previous studies demonstrate conclusively that, under the conditions utilized here, the uptake is optimal, linear with time and tissue concentration, involves specific uptake only into the catecholamine storage vesicles [2, 3, 12, 15, 17], and is comparable to, or better than, uptake seen in more purified preparations [3, 7, 8, 11, 18, 19].

Table 1 shows the results of determinations of [^{3}H]norepinephrine uptake into rat heart, brain, and adrenal vesicles at pH values between 7.4 and 5.7. In all three preparations, uptake declined sharply with decreasing pH, with a 50 per cent reduction occurring by pH 6.6–7.0 and nearly total cessation of uptake by pH 5.7. The uptake profiles showed only minor differences among the tissues studied, providing one indication that, in the rat, the adrenomedullary vesicle is probably a valid model for the study of coupling of energy source to catecholamine uptake into neuronal vesicles.

There are several possibilities which could explain the fall-off in norepinephrine uptake with decreasing pH. First, the ATPase in all three could exhibit a decline in activity over the acidic pH range; however, Taugner [20] has shown that activity of the vesicular Mg^{2+}-activated ATPase is virtually the same as pH values between 6.0 and 8.5. Second, low pH could accelerate catecholamine efflux without altering inward transport, thus leading to an apparent

Table 1. Effect of pH on [^{3}H]norepinephrine uptake into isolated storage vesicles*

Tissue	Percent uptake pH 7.4	7.0	6.6	6.2	5.7
Adrenal	100 ± 4	51 ± 8	20 ± 3	16 ± 5	2 ± 1
Brain	100 ± 3	61 ± 3	50 ± 5	13 ± 3	2 ± 1
Heart	100 ± 4	70 ± 5	24 ± 3	13 ± 2	8 ± 2

* Data are means ± S.E.M. of six to fifteen determinations. Uptakes at pH 7.4 were 2760 ± 110 dpm for adrenal, 9298 ± 280 dpm for brain, and 2692 ± 108 dpm for heart preparations.

decrement in uptake; in fact, low pH values do not appear to affect efflux or may even reduce it [20]. The most likely explanation, then, is that the uptake process in all these preparations is dependent upon a similar proton gradient, an explanation which is supported by the nearly complete attenuation of uptake at pH 5.7, as predicted by the chemi-osmotic hypothesis which indicates an intravesicular pH of 5.5 [9, 10].

In summary, catecholamine storage vesicles isolated from rat heart, brain and adrenal medulla display similar pH profiles for uptake of [^{3}H]norepinephrine. These studies support the view that the mechanism by which energy is utilized indirectly to transport catecholamines into storage vesicles is common to the various tissues containing these transmitters.

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The mechanism of the blockade by trifluoperazine of some actions of phenylephrine on liver and smooth muscle

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The effects of the α-adrenoreceptor agonist phenylephrine on glucose output, O_2 consumption and mitochondrial Ca^{2+} fluxes in perfused rat liver are greatly reduced by the phenothiazine derivative, trifluoperazine [1]. This agent has been reported [2–4] to inhibit the action of calmodulin, a Ca^{2+}-binding protein implicated in intracellular Ca^{2+}-dependent mechanisms (reviewed in [5–7]). Since many of the responses to α-adrenoreceptor activation in a variety of cells (including hepatocytes: see [8, 9]) can be explained as a consequence of a rise in cytosolic Ca^{2+}, the blockade by trifluoperazine of the effects of phenylephrine on perfused rat liver has been considered as possible evidence for the involvement of calmodulin in the actions mediated by α-adrenoreceptors in this tissue [1].

A possible complication is that trifluoperazine is also an α-adrenoreceptor blocking agent, as suggested by experiments with smooth muscle [10, 11] and by measurements of its ability to displace labelled WB4101, a potent α-adrenoreceptor antagonist [12, 13] from binding sites in rat brain [14]. To evaluate this possibility in liver tissue, we have compared the ability of trifluoperazine to block the responses to α-agonists, on the one hand, and to ATP and