The uptake of catechol amines at high perfusion concentrations in the rat isolated heart: a novel catechol amine uptake process

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Commentary by

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This paper resulted from one of the most satisfying experiences which any scientist can have - the accidental discovery of an unexpected phenomenon. In December 1963, towards the end of PhD studies in the Department of Pharmacology in Cambridge under the supervision of Arnold Burgen, I was completing studies on the kinetics of catecholamine uptake in rat isolated heart preparations. Saturation curves for noradrenaline and adrenaline uptake had already been partly completed, indicating half saturation values well below 1 μg/ml for each. For the sake of completeness some experiments were performed at much higher adrenaline concentrations, up to 5 μg/ml. The results showed a much larger than expected accumulation of adrenaline, indicating a rate of uptake that was more than 10 times higher than that predicted from the previous saturation data. As this result was repeated and even higher catecholamine concentrations were explored it rapidly became clear that a wholly different mechanism was operating. The paper described this second transport mechanism, which was termed "Uptake$_2$" for convenience, as a low affinity high capacity transport quite unlike the high affinity low capacity uptake sites in sympathetic nerve endings (Uptake$_1$). Uptake$_2$ was freely reversible, showed a preference for adrenaline over noradrenaline, exhibited little stereochemical selectivity and was relatively insensitive to the Uptake$_1$ inhibitors cocaine and desipramine. The O-methylated catecholamine metabolites normetanephrine and metanephrine were the most potent inhibitors. Although it has subsequently become clear that Uptake$_2$ represents an extraneuronal mechanism, this was not established at the time – and indeed there appeared to be some reduction in its capacity in the small number of sympathetically denervated (immunosympathectomised) hearts available.

Subsequent histochemical studies in a number of laboratories clearly revealed the extraneuronal location of this uptake mechanism. When hearts were perfused with high concentrations of catecholamines a prominent accumulation of amine was observed in the cardiac muscle and other non-neuronal tissues (Ehinger & Sporrong, 1968; Farnebo & Malmfors, 1969; Jacobowitz & Brus, 1971), and this observation was also made in a variety of other peripheral sympathetically innervated tissues. In this context the detailed quantitative histochemical studies performed by John Gillespie and his colleagues on spleen, vas deferens and artery preparations deserve special mention (Gillespie & Muir, 1970; Gillespie, 1973).

Using the rat isolated heart preparation Brian Callingham (Callingham & Burgen, 1966) went on to show that isoprenaline was the preferred catecholamine substrate for Uptake$_2$, and Ullrich Trendelenburg and colleagues in their detailed studies of this mechanism demonstrated that a variety of other monoamines can act as substrates, including 5-hydroxytryptamine and histamine (Grohmann & Trendelenburg, 1984). In my laboratory in Cambridge, Patrick Salt found that Uptake$_2$ was inhibited by corticosterone and various related steroids, and these have proved to be useful research tools (Iversen & Salt, 1970). Stafford Lightman showed that Uptake$_2$ could be demonstrated at much lower perfusion concentrations in the rat isolated heart if the degradative enzymes catechol-O-methyl transferase and monoamine oxidase were inhibited (Lightman & Iversen, 1969). This suggested that Uptake$_2$ was not a threshold phenomenon as at first had

appeared, but operated throughout the range of catecholamine concentrations. At low concentrations no amine accumulation could be observed as the transport amine was rapidly metabolised. These concepts have been refined still further through the meticulous kinetic studies of catecholamine disposition performed by Trendelenburg and colleagues (for review see Bönisch, 1980; Trendelenburg, 1988).

Although the precise function of $Uptake_2$ is hard to define, it represents a widely distributed mechanism in adrenergically sensitive tissues which participates in the disposal of biologically active monoamines - including those released into the circulation from the adrenal medulla and those released from sympathetic nerves. There has been a long standing debate about the relative importance of neuronal uptake versus metabolism via monoamine oxidase and catechol-O-methyl transferase in determining apparent agonist potencies in adrenergically responsive tissues (Kalsner & Nickerson, 1969; Langer & Trendelenburg, 1969; Trendelenburg, 1973; Iversen, 1971, 1975). The answer can now be seen to depend on which tissue is being studied and the relative affinities of the agonist for neuronal versus extraneuronal uptake. In such tissues as vas deferens or cat nictitating membrane which have a dense sympathetic innervation (i.e. a high capacity of $Uptake_1$ sites) the neuronal uptake mechanism is far more important than in such tissues as aorta or other arteries, in which the neuronal uptake sites are more sparsely distributed and released catecholamine may travel significant distances from sites of neural release to smooth muscle targets (Trendelenburg *et al*, 1969; Burnstock *et al*, 1972). In densely innervated tissues the dose-response curves for noradrenaline and adrenaline will usually show a leftward shift in the presence of cocaine or desipramine, whereas in sparsely innervated tissues inhibitors of $Uptake_2$ (e.g. steroids) or inhibitors of catechol-O-methyl transferase will have this effect whereas cocaine and desipramine are ineffective (Kalnser, 1969; Kaumann, 1972).

Recent years have seen further progress in research on the extraneuronal transport mechanism. $Uptake_2$ may well correspond to the organic cation transporter (OCT1) recently cloned from kidney (Gründemann *et al*, 1994). Schömig and colleagues in Würzburg have identified a human cell line (Caki-1) as a convenient model system for studying $Uptake_2$ (Schömig & Schönfield, 1990), and they have shown that the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) is a substrate (Russ *et al*, 1992). This group has also identified a series of cyanine dye derivatives that act as inhibitors of $Uptake_2$ at nanomolar concentrations (Russ *et al*, 1993).

Very recently it has been reported that $Uptake_2$ is expressed in human glioma cell lines (Streich *et al*, 1996) and that the sensitivity of some glioma cells to nitrosourea derivatives may depend on the selective accumulation of these drugs into the cells via the $Uptake_2$ mechanism (Noë *et al*, 1996). The possibility of designing new chemotherapeutic agents that are selectively targeted to tumours by means of $Uptake_2$ appears to be yet another unexpected discovery in this line of research.

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