$Uptake_2$ relative to $Uptake_1$. This is because 3-methoxyisoprenaline is 169 times, while normetanephrine is only 15 times, more potent in inhibiting $Uptake_2$ than $Uptake_1$.

The results of Burgen & Iversen (1965) show that in the rat heart normetanephrine was 48 times, while metanephrine was 15 times, more potent in inhibiting $Uptake_2$ than $Uptake_1$. The IC 50 for normetanephrine on $Uptake_1$ was $1{\cdot}75 \times 10^{-4}$ M in this study which is similar to the value of 2×10^{-4} M obtained by Burgen & Iversen (1965) in the rat heart. Jarrot (1970) has shown that the affinity of noradrenaline for the neuronal uptake site in the perfused mouse heart is similar to that in the rat, so it seems reasonable to combine the results of Burgen & Iversen with those of this study in order to obtain an idea of the selectivity of metanephrine relative to normetanephrine and 3-methoxyisoprenaline. This suggests that the order of increasing selectivity for $Uptake_2$ in preference to $Uptake_1$ is metanephrine $<$ normetanephrine $<$ 3-methoxyisoprenaline.

The greater selectivity of 3-methoxyisoprenaline for $Uptake_2$ than $Uptake_1$ may allow its use as a pharmacological tool in experiments in which inhibition of $Uptake_2$ is required but $Uptake_1$ must be relatively uneffected.

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Monoamine tetrazolium reductase of rat heart mitochondria

The presence of monoamine dehydrogenase (MADH) in rat brain and liver suspensions has been reported earlier through the use of tetrazolium salts as hydrogen acceptors (Lagnado & Sourkes, 1956). Studies indicating a dissimilarity of rat brain MADH from monoamine oxidase (Guha & Ghosh, 1970) led us to investigate the presence of MADH in rat heart mitochondria and study some of its properties.

Heart homogenate and isolated mitochondria from albino rats (200–300 g) were prepared in cold 0·25 M sucrose (Sen, Parmar & Guha, 1968). The reaction mixture for determination of MADH activity consisted of 0·025 M tris-HCl buffer, pH 8·0, 0·5 mg neotetrazolium chloride (NTC), 0·01 M of the desired substrate and tissue homogenate or isolated mitochondria equivalent to 100 mg wet tissue weight in a total volume of 2 ml. The reaction mixture containing heart mitochondria was incubated at 37° for 10 min. After the addition of the desired substrate, the mixture was further incubated for 30 min using air as a gas phase for aerobic conditions whereas anaerobic experiments were carried out in a vacuum in Thunberg tubes (Guha & Ghosh, 1970). MADH activity was determined by estimating at 520 nm the red diformazon formed as a result of NTC reduction (Lagnado & Sourkes, 1956). Monoamine oxidase

Table 1. *Effect of potassium cyanide, semicarbazide and monoamine oxidase inhibitors on rat heart mitochondrial monoamine dehydrogenase system.* Each experiment was done in duplicate and the values are mean of three separate experiments. Assay conditions for MADH activity are same as described in the text. Aliquots of the mitochondrial suspension used as the source of enzyme and the inhibitors were incubated for 15 min at 37° before the addition of tryptamine. Inhibition or activation is expressed relative to the sample of the enzyme without additions.

Additions	Concentration (M)	Effect on MADH activity	
		Inhibition (%)	Activation (%)
Potassium cyanide	1×10^{-3}	50·0	—
Semicarbazide	$1{\cdot}25 \times 10^{-2}$	no effect	no effect
	2×10^{-2}	—	24·5
	4×10^{-2}	—	31·7
Iproniazid	1×10^{-3}	84·4	—
Pheniprazine	1×10^{-3}	42·0	—
Nialamide	2×10^{-3}	43·7	—
Pargyline	1×10^{-5}	no effect	no effect
	1×10^{-3}	no effect	no effect
Tranylcypromine	1×10^{-5}	no effect	no effect
	1×10^{-3}	no effect	no effect

inhibitors, potassium cyanide and semicarbazide were incubated with isolated heart mitochondria for 10 min before the addition of the desired substrate. All values were corrected for appropriate enzyme and substrate blanks.

The maximum MADH activity was observed when adrenaline was used as the substrate where the amount of diformazon formed was 121·9 μ mol g^{-1} of wet heart weight. On the other hand, 57·5, 34·4, 16·2 and 3·8 μ mol of diformazon g^{-1} of wet tissue weight was formed when tryptamine, 5-hydroxytryptamine, tyramine and noradrenaline were used as substrates, respectively (figures are means of 3 separate experiments each made in duplicate). Our results have indicated definite decrease in MADH activity under anaerobic condition since only 7·5 μ mol g^{-1} of wet heart weight was formed from tryptamine anaerobically, thereby exhibiting 87% reduction in MADH activity. Low MADH activity under anaerobic conditions could possibly account for the high endogenous reduction of NTC observed in these experiments. Such a decrease in MADH activity was found to be specific for heart mitochondria since anaerobiosis has been shown earlier to increase MADH activity of rat brain homogenate (Guha & Ghosh, 1970). Selective inhibition, amongst monoamine oxidase inhibitors, by iproniazid alone in concentrations far in excess of those required for monoamine oxidase inhibition, has provided evidence towards dissimilarity between heart MADH and monoamine oxidase. These observations were further supported by pH optimum of 8 observed for MADH as compared to pH 7·0 for rat heart mitochondrial monoamine oxidase, and also by inhibition of MADH by potassium cyanide and stimulation by semicarbazide (Table 1).

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More on the blockade of neural and exogenous noradrenaline in vascular tissue

Recently published data from our laboratory do not support the traditional viewpoint on blockade of responses to exogenous and neural noradrenaline by α-adrenoceptor blocking agents (Wyse & Beck, 1972). It is generally accepted that responses to exogenous amine are more readily blocked by α-adrenoceptor blocking agents. Our data (Wyse & Beck, 1972) show that responses of small arteries from dog mesentery to exogenous and neural noradrenaline are blocked to an equivalent degree by low doses of phenoxybenzamine. Bevan, Su & Ljung (1973), have questioned our conclusion and in this paper we reply to the points they raised and present further data to reaffirm our original conclusion.

In vivo investigations by Levin & Beck (1967) and Miranda & Gomez (1970) have unequivocally demonstrated that sympathetic α-adrenoceptor responses of the hindlimb vasculature are more readily blocked by α-adrenoceptor blocking agents than are equivalent responses to injected amine. These findings were not noted by Bevan & others (1973) in their discussion of our data. Our *in vitro* experiments attempt to approximate to the *in vivo* situation by the use of small, densely innervated resistance blood vessels.

In the present experiments the methods were identical to those earlier outlined (Wyse & Beck, 1972) with the following differences. Experimental tissues were helical strips of rat ventral tail artery (0·7 to 0·9 mm o.d.). Square wave pulses of supramaximal current strength were delivered from a Grass Model S48 stimulator with a low output impedance (25 ohm). Both the "field" voltage (approximately 15 V) and the output current (calculated from voltage across a 1 ohm resistor) were monitored on an oscilloscope. Frequency-response curves were obtained by delivery of a 30 s train of pulses at selected frequencies with a 2 min pause between each stimulus during which the bath media were not changed. These parameters were chosen by experimentation as those giving maximal responses at each frequency without significant deterioration over several hours.

Final concentrations of noradrenaline are expressed as nmol (w/v) of the base litre^{-1}. Final concentrations of phenoxybenzamine (courtesy of H. A. Sheppard, Smith, Kline & French) are expressed as mol (w/v) of the hydrochloride salt litre^{-1}.