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KINETICS OF NOREPINEPHRINE METABOLISM IN THE RAT HEART *IN VIVO*

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Abstract—Concentrations of labeled norepinephrine and its metabolites were measured under steady-state conditions in the rat heart. From these values, rate constants of a simplified model of norepinephrine metabolism were calculated. It was found that 90 per cent of norepinephrine and 66 per cent of normetanephrine were metabolized by monamine oxidase, while 10 per cent of norepinephrine and 27 per cent of deaminated metabolites were metabolized by catechol-*O*-methyltransferase. The release of unchanged norepinephrine from the heart was smaller than the uptake of labeled norepinephrine from the circulation into the heart. These results indicate a significant role of peripheral nerve endings for the sympathetic system in the heart and a possible source of error for measurement of norepinephrine turnover in the heart.

IT IS GENERALLY accepted that in the heart, as well as in other tissues with adrenergic innervation, norepinephrine (NE) is metabolized mainly intraneuronally by monoamine oxidase (MAO) and much less extraneuronally by catechol-*O*-methyltransferase (COMT).[1,2] However, it is not known which exact proportion of NE is converted by each of these enzymes. Because both MAO and COMT act on the product of the other enzyme, measurements of NE metabolites in the heart at one point after administration of labeled NE[3] or measurements of metabolite efflux from the heart *in vitro*[4] do not provide direct information about the sequence in which MAO and COMT participate in NE metabolism.

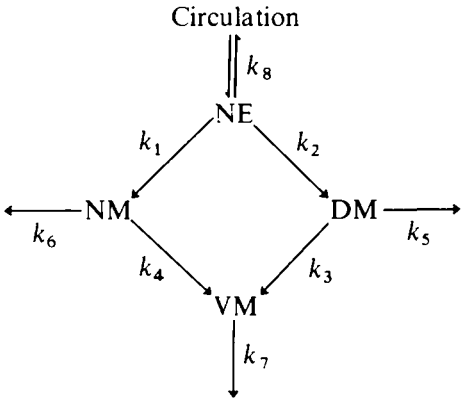
In this study we attempted to determine the sequence and extent of MAO and COMT action by a kinetic analysis of NE and its metabolites in the hearts of rats pretreated with labeled NE.

METHODS

Determination of ^{3}*H-NE and its* ^{3}*H-metabolites.* Sprague–Dawley male rats, weighing 230–270 g, were injected intravenously with 2·27 μg/kg of ^{3}H-NE (*dl*-norepinephrine-7-^{3}H, sp. act. 7·44 Ci/m-mole; New England Nuclear) and were sacrificed 24, 32, 40 or 48 hr later by cervical dislocation. Five rats were used for each time point. The hearts were dissected, blotted and homogenized immediately in 4 vol. of cold 0·4 N perchloric acid (containing 1 ml of 10% Na_2-EDTA and 1 ml of 25% Na_2SO_3 in 100 ml $HClO_4$) by an Ultra-Turrax steel homogenizer. The supernatant was adjusted to pH 4–5 by KOH, and the samples were stored frozen. Norepinephrine and its metabolites were separated according to the method of Leitz and Stefano.[4] Separation of *O*-methylated compounds and catechols on an alumina column was followed by separation of amines and acid compounds present in each fraction by passage through the Dowex 50 column. By this method we obtained four fractions: a

fraction containing NE (NEF), a fraction containing normetanephrine (NMF), a fraction containing 3,4-dixydroxymandelic acid and 3,4-dihydroxyphenylglycol (DMF), and a fraction containing 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol (VMF). The radioactivity of each of these fractions was determined by a liquid scintillation counter and corrected for recovery and quenching.

Calculation of the rate constants. Kinetic analysis of the data was performed using a simplified model of NE metabolism, assuming that all metabolic steps are first-order processes:*



The levels of labeled NE and its metabolites are described by the following set of differential equations:

$$dNE/dt = -k_A NE \quad \text{where} \quad k_A = k_1 + k_2 + k_8 \tag{1}$$

$$dDM/dt = k_2 NE - k_B DM \quad \text{where} \quad k_B = k_3 + k_5 \tag{2}$$

$$dNM/dt = k_1 NE - k_C NM \quad \text{where} \quad k_C = k_4 + k_6 \tag{3}$$

$$dVM/dt = k_3 DM + k_4 NM - k_7 VM \tag{4}$$

These equations were solved using Laplace transformation:

$$NE_t = NE_0 e^{-k_A t} \tag{5}$$

$$DM_t = \frac{k_2 NE_0}{k_B - k_A}(e^{-k_A t} - e^{-k_B t}) \tag{6}$$

$$NM_t = \frac{k_1 NE_0}{k_C - k_A}(e^{-k_A t} - e^{-k_C t}) \tag{7}$$

$$VM_t = -k_2 k_3 NE_0 \frac{(k_3 - k_7)e^{-k_A t} + (k_7 - k_A)e^{-k_3 t} + (k_A - k_3)e^{-k_7 t}}{(k_A - k_3)(k_3 - k_7)(k_7 - k_A)}$$
$$-k_1 k_4 NE_0 \frac{(k_4 - k_7)e^{-k_A t} + (k_7 - k_A)e^{-k_4 t} + (k_A - k_4)e^{-k_7 t}}{(k_A - k_4)(k_4 - k_7)(k_7 - k_A)} \tag{8}$$

and the rate constants were calculated as described in the Results.

* NM = normetanephrine; DM = 3,4-dihydroxymandelic acid; VM = 3-methoxy-4-hydroxymandelic acid.

RESULTS

In a separate experiment it was determined that after administration of 2·27 μg/kg of ^{3}H-NE, the decline of labeled NE between 24 and 48 hr is exponential (Fig. 1). It was possible, therefore, to calculate the apparent initial level of labeled NE (NE_0) and the apparent rate of NE disappearance (k_A) from the data in Table 1 (NEF) using equation (5). We obtained 39,850 dis./min for NE_0 and 0·038 hr^{-1} for k_A.

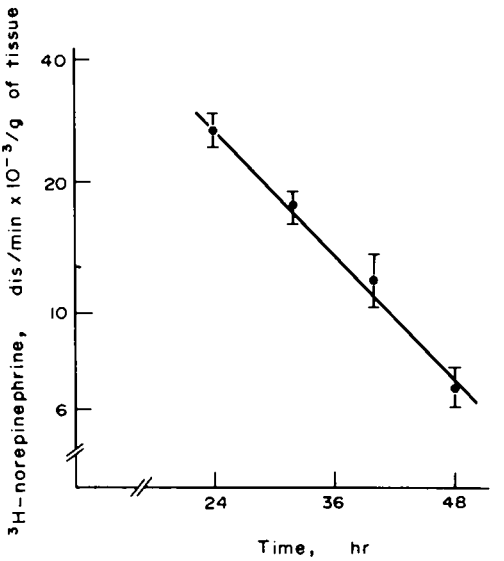


FIG. 1. Decline of ^{3}H-norepinephrine from the rat heart. The bars indicate S.E.M.

Equation (6) was then formulated for $t = 24$ and $t = 48$ hr. By combination of these two equations, we eliminated the rate constant k_2 and obtained the equation:

$$DM_{24}e^{-48k_B} - DM_{48}e^{-24k_B} + (DM_{48}e^{-24k_A} - DM_{24}e^{-48k_A}) = 0. \quad (9)$$

Into this equation we substituted values for DMF_{24} and DMF_{48} from Table 1 and the value of k_A calculated above. The equation was then solved as a quadratic equation with $x = e^{-24k_B}$. One of the roots of this equation was very close to k_A (0·0381 hr^{-1}) and therefore could be eliminated. The second root indicated that the value of $k_B = 0·122\ hr^{-1}$. This value was then substituted into equation 6 and the equation was solved for k_2 (0·0466 hr^{-1}).

TABLE 1. ^{3}H-NOREPINEPHRINE AND ITS METABOLITES IN THE RAT HEART*

	24 hr	48 hr
Norepinephrine (NEF)	15,972 ± 2,324	6,430 ± 693
Normetanephrine (NMF)	1,179 ± 244	620 ± 243
3,4-Dihydroxymandelic acid and 3,4-dihydroxyphenylglycol (DMF)	7,708 ± 878	3,497 ± 415
3-Methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol (VMF)	15,930 ± 1,097	13,232 ± 1,880
Total activity	40,770 ± 4,057	23,423 ± 1,992

* Values are expressed as dis./min/g of tissue ± S.E.M.

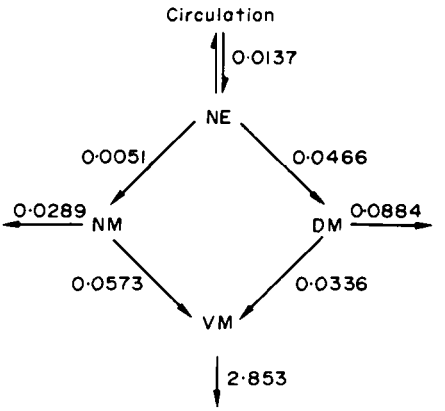


FIG. 2. Simplified model of norepinephrine metabolism with appropriate rate constants. The arrows indicate net flow of labeled substances.

Equation (7) was used in a similar manner for calculation of rate constants k_1 and k_C. The values of NMF from Table 1 and the value of k_A from above were substituted into an equation analogous to equation 9. One root of this equation was also very close to k_A (0·0381 hr^{-1}) and the second indicated the value of k_C to be 0·0862 hr^{-1}. Substituting this value into equation (7) we obtained 0·0051 hr^{-1} for k_1. Using equation (1) it was then possible to calculate the rate constant k_8:

$$k_8 = k_A - (k_1 + k_2). \tag{10}$$

We obtained $-0{\cdot}0137\ hr^{-1}$ for k_8.

For calculation of rate constants k_3, k_4 and k_7, we used digital computer iteration. We designed a program which solved equation (8) for both $t = 24$ and $t = 48$ and con-

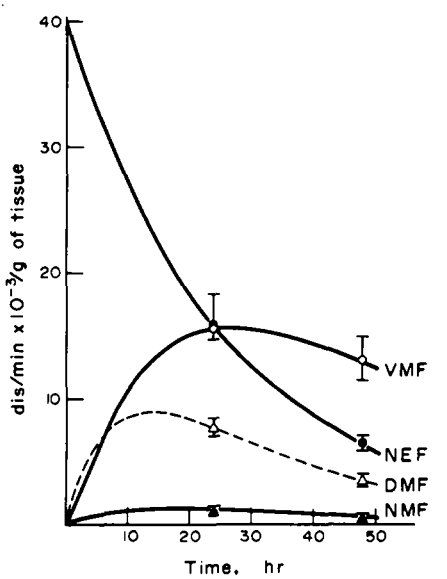


FIG. 3. Concentrations of labeled norepinephrine and its metabolites calculated from the simplified model. The points indicate experimental values from Table 1.

verted the results into per cents of the respective VMF values. As the initial condition, we substituted k_C for k_3 and k_B for k_4 because, according to our model, $k_3 \leq k_C$ and $k_4 \leq k_B$. Initial condition of $k_7 = 3{\cdot}0\ hr^{-1}$. Values of these rate constants were independently gradually decreased, and the computer printed out only combinations which, when substituted in equation (8), provided a solution which did not differ considerably from 100 per cent for both time intervals. In the first two steps, the values of the rate constants were narrowed down to $0{\cdot}037 > k_3 > 0{\cdot}0325$, $0{\cdot}061 > k_4 > 0{\cdot}029$ and $2{\cdot}86 > k_7 > 2{\cdot}84$. In the following step, it was found that the combination of rate constants which best fitted the equation was $k_3 = 0{\cdot}0336\ hr^{-1}$, $k_4 = 0{\cdot}0573\ hr^{-1}$, and $k_7 = 2{\cdot}853\ hr^{-1}$. (VMF values were 100·002 and 99·997 per cent respectively.)

Figure 2 shows our model with rate constants calculated as described above. Levels of labeled NE and its metabolites calculated from the rate constants and the actual experimental points are shown in Fig. 3.

DISCUSSION

In order to calculate the rate constants of NE metabolism, we had to make certain assumptions. The first of these assumptions was that all the steps involved in NE metabolism are first-order processes. It was shown by Neff *et al.*[5] that the labeled NE after injection of a tracer dose of ^{3}H-*l*-NE disappears from the heart by simple exponential decay. In the present experiments we used a higher than tracer amount of ^{3}H-NE to permit measurement of NE metabolites. It was reported that after a dose of NE similar to that used in our experiments the disappearance of labeled NE was biphasic, with the first phase lasting about 12 hr.[5] Since we started our determinations 24 hr after injection of NE, the decline of labeled NE was observed as monophasic (Fig. 1) and represented the physiological turnover of heart NE.[6] Racemic labeled NE was used in our experiments, but the decline observed between 24 and 48 hr after injection was due almost exclusively to the presence of *l*-NE, since the *d*-form disappears much more rapidly ($T_{1/2} = 3{\cdot}75$ hr).[7]

Formation of NE metabolites depends on the rate of NE diffusion to the enzyme site. The activity of metabolizing enzymes is apparently, under normal circumstances, not a rate-limiting factor, since drugs affecting storage and/or release of NE increase considerably the formation of NE metabolites. It was shown that release of NE induced by tyramine increases the formation of normetanephrine several-fold, while release of NE induced by reserpine increases formation of deaminated metabolites.[8] The rate of metabolism of normetanephrine and deaminated catechols depends also on their diffusion to the respective enzyme, since MAO and COMT are localized at different parts of the synaptic complex.[2]

In our simplified model we combined acid metabolites and their respective glycols and treated them as one compartment. We also did not consider intermediate aldehyde formation during deamination. Therefore our rate constants for deamination reflect either the combination of or the slower of the rate constants of both the deaminating and oxidative steps. The rate constant for VM formation from DM includes formation of both 3-methoxy-4-hydroxymandelic acid from 3,4-dihydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol from dihydroxyphenylglycol. Since both of these conversions are catalyzed by the same enzyme, we consider this to be an acceptable simplification.

Rate constants estimated from our data provide information about the proportion of NE and its metabolites converted by MAO, COMT or released from tissue. The ratio of rate constants for two parallel pathways reflects the relative degree of formation of metabolites by each pathway. Therefore from the ratio of rate constants k_1 and k_2 the proportion of NE which is deaminated or methoxylated can be calculated. Similarly it can be calculated from the respective rate constants which proportion of metabolites is released from the tissue and which is further metabolized. Table 2 summarizes the results obtained by this calculation. It can be seen that in the rat heart about 10 per cent of NE is metabolized by COMT and the rest by MAO. About one quarter of deaminated catechols is further metabolized by COMT and two-thirds of normetanephrine are metabolized by MAO.

TABLE 2. PROPORTION OF NOREPINEPHRINE AND ITS METABOLITES CONVERTED BY MONOAMINE OXIDASE, CATECHOL-*O*-METHYLTRANSFERASE OR RELEASED FROM THE TISSUE

	Monoamine oxidase (%)	Catechol-*O*-methyltransferase (%)	Released (%)
Norepinephrine	90·14	9·86	*
3,4-Dihydroxymandelic acid and 3,4-dihydroxyphenylglycol		27·54	72·46
Normetanephrine	66·47		33·53

* Release of norepinephrine is compensated for by uptake of norepinephrine from the circulation.

From the proportion of NE metabolized by each of the pathways and from the proportion of metabolites released unchanged into the circulation, the relative proportion of all NE metabolites released from the heart can be calculated. It was calculated that NE metabolites consist of 3·3 per cent normetanephrine, 65·3 per cent 3,4-dihydroxymandelic acid and 31·4 per cent 3-methoxy-4-hydroxymandelic acid. These values are in good agreement with the proportion of metabolites released during heart perfusion *in vitro*.[4] The main difference between results *in vitro* and *in vivo* is that according to our calculations there is no net release of unchanged NE from the heart *in vivo*. The rate constant k_8, which is a combination of rate constants for uptake of NE from the circulation and for the efflux of NE from the heart, was a negative number. This indicates that the influx of labeled NE into the heart during our observation period was greater than efflux of unchanged NE from the heart.

It is known that the heart has a higher uptake capacity for NE and that the main source of circulating NE is probably nerve endings in the blood vessels.[9] The heart therefore is able to extract efficiently NE released into the circulation from peripheral nerve endings. Since after i.v. isotope administration these nerve endings were also labeled, ^{3}H-NE was released from them into the circulation and taken up into the heart; this process decreased the rate of disappearance of ^{3}H-NE from the heart. By comparison of the fractional rate constants and the apparent rate of disappearance, it was calculated that over 25 per cent of NE metabolites originated from NE taken into the heart from the circulation. If some unchanged NE was released, as was found in the experiment *in vitro*, the fraction of NE taken up from the circulation might be even larger. On the other hand, it is possible that our calculations were influenced by metabolites formed during the first phase of ^{3}H-NE decline which remained in

the heart. It was reported, however, that during the first phase NE is released mostly unchanged or as normetanephrine.[10] Our measurements started 12 hr after the end of the first phase, and it is therefore unlikely that a significant amount of metabolites from the first phase was still present in the heart.

Our calculations could be influenced by an uptake of NE metabolites into the heart from the circulation. Normetanephrine is taken up from the perfusion medium, but it is washed out rapidly[11] and only a small amount of normetanephrine is present in the circulation.[12] The uptake of deaminated metabolites into the heart is unlikely, since it was reported that the volume of distribution of ^{3}H-3-methoxy-4-hydroxymandelic acid is much smaller than the extracellular space.[13]

The uptake of NE released from the peripheral nerve endings can partly explain why the rate of loss of labeled NE in isolated hearts is significantly faster than the decay of labeled NE from the heart *in vivo*.[14,*] The presence of significant uptake of NE from peripheral sources into the heart also indicates that calculation of the turnover rate of heart NE from the decay of labeled NE[15] underestimates the turnover of NE in the heart. Interaction of the heart and the peripheral adrenergic nerve endings may play a role in the action of some drugs or in some pathological conditions and it warrants further investigation.

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