Chronotropic Effects of Norepinephrine and Tyramine: A System Response Analysis

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In 1958, Burn and Rand proposed that the drug, tyramine, had no direct sympathomimetic effects and that its pharmacologic action was wholly mediated by the liberation of norepinephrine from endogenous stores. This was based upon the observation that preparations severely depleted of stored norepinephrine by pretreatment with reserpine responded poorly, if at all, to tyramine. Large amounts of norepinephrine infused into such preparations prior to tyramine administration restored the potency of the latter amine and this was interpreted as the result of storage site repletion.

Such repletion of storage site with norepinephrine does not seem to be a necessary condition for the restoration of tyramine efficacy. Certain investigators have concluded that the presence of proportionately small amounts of unbound extracellular norepinephrine is sufficient to enhance tyramine effect. These authors argue that tyramine must have an effect different from, and in addition to, the simple release of norepinephrine from storage and that this superadded effect is functionally related to the presence of unbound norepinephrine.

Direct evidence for the release of norepinephrine from organs responding to tyramine is abundant. However, the amounts of norepinephrine liberated by tyramine are small compared to those needed to achieve the same pharmacologic response by administration of exogenous norepinephrine. The time course of sympathomimetic response to an injectate of tyramine is protracted as compared to an injectate of norepinephrine at least in its inotropic and chronotropic effects upon the canine heart-lung preparation. This suggests a capacitative effect upon the norepinephrine released by tyramine; that its rate of decay of concentration in the region of the receptors is much slower than would be predicted by elutional processes. This might be explained by some mechanism of slow release of norepinephrine by tyramine. If this were the mechanism responsible one must then invoke the hypothesis of "strategic liberation" to explain profound pharmacologic effects by small amounts of liberated norepinephrine. This type of hypothesis has led to the proposal that local liberation of norepinephrine near the S-A node is a possible explanation for the chronotropic effects of tyramine, but this ignores the concomitant rate independent, inotropic effect on the whole cardiac muscle mass. "Strategic liberation" theories do not explain the potentiation of tyramine effect by unbound norepinephrine unless one predicated a nonsymmetric ingress-egress pathway for norepinephrine resulting in tyramine liberating norepinephrine from strategic sites but not blocking its access to those sites.

It is the purpose of this paper to propose a kinetic hypothesis consistent with the observed interaction between the two amines in question. In essence, the proposal of Burn and Rand concerning tyramine mechanism is accepted. No direct effect is attributed to tyramine. The release of norepinephrine from storage site is considered to be mediated by a shift of a dynamic equilibrium between free and bound norepinephrine effected by the competition of tyramine with norepinephrine for free storage sites. A concentrating
mechanism is proposed between intravascular space and "receptor site space," i.e., the site of action of norepinephrine. This is viewed as producing a mass partition ratio greatly favoring receptor site space. This would allow accumulation of tyramine liberated norepinephrine in receptor site space so long as the concentrating mechanisms were highly specific for norepinephrine, thereby disallowing tyramine competitive inhibition. Receptor site space as considered in this formulation is an operationally defined mathematical space and cannot be precisely delimited anatomically on the basis of the present study. While it may be viewed as being in extracellular space it cannot be assumed with any certainty to be identical with that space.

In such a system injected norepinephrine effect would be rate-limited by the availability of storage sites for uptake from receptor-site space. Tyramine would have no effect in a system devoid of norepinephrine. However, tyramine may still occupy storage sites and in the absence of significant amounts of norepinephrine might partially saturate those sites. Norepinephrine arriving subsequent to this blockade would be removed relatively slowly from receptor-site space and would have a longer and greater response. It need not be presumed that this space have a great mass capacitance and thus uptake in denervated preparations, i.e., without storage sites, might not be significant.

Methods

Isolated heart-lung preparations were prepared from mongrel dogs in the usual manner employing ether-chloralose (1 g iv) anesthesia. The superior vena cava and brachiocephalic artery were cannulated and other major systemic vessels ligated. A Starling resistor in the arterial outflow was set at an occlusion pressure of approximately 100 mm Hg. Blood circulated through a temperature regulated external reservoir initially filled with heparinized blood removed from donor dogs under ether anesthesia just prior to the experiment. When done, coronary sinus cannulation was performed with a polyethylene cannula passed via the right atrial appendage into the ostium of the coronary sinus and secured with an encircling suture of 4-0 silk.

Experimental dogs were reserpinized by intraperitoneal injection of 0.5 mg reserpine U.S.P. 48 hours and 0.75 mg 24 hours prior to the experimental procedure.

Labelled amines used in this study were \( \text{i-d} \)-norepinephrine-2-C\(^{14}\) and \( \pi \)-hydroxyphenylethylamine-1-C\(^{14}\) (tyramine). Whole blood concentrations of these compounds were estimated by proportional counting of \( \beta \) activity of air-dried whole blood aliquots on aluminium planchets. Standards were prepared for each set of determinations and calibration curves were satisfactorily linear in the range of interest.

Cardiac rate was monitored on a digital cardiotachometer with an output for analog recording. The "arterial" pressure pulse in the outflow tubing from the preparation was used as the triggering voltage for the tachometer. Recordings were made on a Sanborn six-channel paper strip recorder.

Electronic simulations were constructed on the problem boards of two Donner 3400 analog computers employing model 3732F quarter square multipliers for multiplication and power functions and a model 3750 variable base function generator for the simulation of empirically determined forcing functions.

Results

**STEADY STATE CALIBRATION**

Carbon-14 norepinephrine was infused into the inflow tubing of heart-lung preparations and allowed to recirculate through a small volume while the heart rate was continuously monitored and recorded. A plateau of constant rate was established between one and two minutes after addition. During rate equilibrium two consecutive ten-second aliquots of blood were removed from the arterial cannula and considered representative of blood entering the coronary circulation. Norepinephrine concentration was measured as C\(^{14}\) activity. The criterion of steady state norepinephrine input was that the difference between the paired aliquots be no greater than three times the standard error of the method of estimation, i.e., the samples differed by no more than 13.2%. The value was omitted from consideration if this criterion was not fulfilled. Immediately after sampling additional norepinephrine was administered and the procedure repeated. No more than six additions were made in any preparation so that all values were obtained within twelve minutes after the first addition. Blood that had been re-
moved from the reservoir prior to the additions of norepinephrine and kept at the same temperature was returned to the reservoir and samples were taken when rate equilibrium was established. The values obtained on decreasing concentrations are not included in the graphed data. There was no difference in the range of values obtained immediately in this manner and the rate-concentration relationship obtained during the period of increasing concentrations. The absence of significant hysteresis in the system, thus demonstrated, was important in the formulation of the dynamic response analysis given below and gives reasonable assurance that the C$^{14}$ label largely represented the originally labelled molecular species during the time of the study.

Sixteen points were obtained from four preparations from reserpine pretreated animals in this manner. The coronary sinus was cannulated in two of these preparations and the efflux excluded from recirculation. The diversion of the coronary outflow did not affect the concentration-rate relation in any evident manner. There was approximately 50% reduction in C$^{14}$ activity in the coronary sinus blood as compared to aortic root blood activity. Rate increase over base line levels was plotted against aorta root norepinephrine concentration, yielding the array of points seen in figure 1.

Because of the distribution of the points and because there is no a priori theoretic reason to necessitate the use of another function, the positive limb \( y = A \sqrt{x} \) of a parabola of the form \( y^2 = A^2 x \) was chosen to represent in continuous form the functional relationship between rate increase and norepinephrine concentration. A least squares fit gave a value of 10.24 for the coefficient \( A \); the coefficient of variation of the points was 18.0%, equivalent to a standard deviation of \( A \) of ± 1.84. Iteration on increments to the origin of the point array in each dimension was performed on an IBM 650 digital computer with

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\text{Rate increase in beats per minute above base line rate plotted against the concentration of C$^{14}$ norepinephrine in the aortic root. Values were obtained under steady state conditions in preparations from reserpine pretreated dogs. Dashed line represents the approximating function: rate increase equals 10.24 times the square root of norepinephrine concentration.}
\]

\[
\text{FIGURE 1}
\]

\[
y = 10.24 \sqrt{x}
\]

\[
\text{Rate increase plotted against C$^{14}$ norepinephrine aortic root concentration. Solid points represent the values obtained from reserpinized hearts as in figure 1. Open circles represent values from normal hearts with an increment of 18 beats per minute and 3.6 µg/liter norepinephrine concentration on each. Arrow indicates the translated origin of the point array from normals. Dashed line is the approximating function; rate increase equals 9.48 times the square root of C$^{14}$ norepinephrine concentration.}
\]

\[
\text{FIGURE 2}
\]

\[
y = 9.48 \sqrt{x}
\]
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least squares fit and variance estimate for each iteration to determine if a portion of a parabolic limb not containing the vertex might give a better approximation. No better fit was found. The approximating function, rate increase equals $10.24 \times \sqrt{\text{norepinephrine concentration}}$, is shown in figure 1.

Nineteen points were obtained from five heart-lung preparations from normal dogs. Division of coronary sinus flow apparently did not alter the response pattern in the two dogs in which this was done. A similar extraction ratio of label between the aortic and coronary sinus blood was found in these hearts as was found in the reserpine pretreated hearts. The array of points from these preparations fell well below the points from reserpinized dogs when plotted in a like manner. The best fitting parabolic limb including the vertex had a value for the coefficient $A$ of 6.2. The origin of this array was translated along the previously determined approximating function until all but two points lay within the range of two standard deviations of the original $A$. This resulted in an initial state offset of approximately 3.6 $\mu$g/liter norepinephrine concentration for normal values. Considering all points, a predicting function resulted with a value of coefficient $A$ of 9.48, differing by 0.4 standard deviation from the original and with a coefficient of variation of 20%. Using this relation, the initial state offset for normal values was 18 beats per minute. The combined array of points and the approximating function, rate increase equals $9.48 \times \sqrt{\text{norepinephrine concentration}}$, are shown in figure 2.

RESPONSE TO TRANSIENTS

The study of the system response to transient increases of norepinephrine concentration was now undertaken. Injections of labelled norepinephrine were made rapidly into the inflow tubing of the heart-lung preparations. The whole blood concentration of norepinephrine as a time function was estimated at the aortic root by interrupted sampling at 2.25-second intervals and appropriate counting. The heart rate was continuously recorded during this time as an analog curve. Thus input and output (response) of the system were available as time functions.

The curve of the input concentration was approximated by linear segments of an electronic function generator and used as the forcing function of an analog computer circuit (fig. 3A). The square root mode of a quarter-square multiplier with proper attenuation was used to generate the nonlinear calibration curve relationship. One $\mu$g/liter norepinephrine concentration and one beat per minute rate change were each scaled to one volt. Time scaling was done for rapidity of repeated solution. Suitable initial state voltages were supplied for simulation of normal ($G(0) = 3.6$ V) and reserpine pretreated ($G(0) = 0$ V) preparations. A single computer unit-lag circuit was found satisfactory to trans-

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**FIGURE 3**

Circuit A represents the analog computer configuration for solution of equations 1 and 2. See text. Circuit B represents the computer configuration for solution of equations 3, 4, and 5. QSM designates a quarter-square-multiplier in square root mode whose output is 10 V input. As shown simulation is in real time.

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form the forcing function into a good representation of the rate response displayed according to the calibration transformation. The unit-lag circuit solves a first order differential equation with unity gain.

Repetitive solution was performed and the best fit chosen by visual match on an oscilloscope face. The only variable system parameters were the coefficient A of the calibration transformation and the time constant of the unit-lag circuit. These could be varied by changing potentiometer settings.

The equations describing the simulating circuitry are:

\[ G(t) = F(t) - \left( \frac{1}{\alpha} \right) (dG/dt) \] (1)

and

\[ R(t) = A \sqrt{G(t)} + G(0) - R(0) \] (2)

where

- \( F(t) \) = Aortic root norepinephrine concentration time function
- \( R(t) \) = Rate increase above base line rate
- \( G(0) \) = Initial displacement on calibration curve in \( \mu g/\text{liter} \)
- \( R(0) \) = Initial rate displacement = \( A \sqrt{G(0)} \)
- \( G(t) \) = A time function defined in the first equation above
and \( 1/\alpha \) = The natural time constant of the unit-lag circuit.

The correspondence between computer predicted response and experimental results is seen in figure 4. The values of time constants that yielded good fits were between five and thirteen seconds. A quantitative description of the norepinephrine input-response transfer relation is thus available and must be satisfied by the general system model.

The system response to transient increase of tyramine concentration was now established by a similar method of aortic root sampling and rate monitoring. Because of the "cumulative" type of tyramine response to transients, it is clear that no steady state calibration could be performed as was done for norepinephrine. Assuming that tyramine effect was norepinephrine mediated, the norepinephrine static calibration transform was used as the last stage in the tyramine computer simulation.

\[ G(t) = (B \times F(t)) - \left( \frac{1}{\alpha} \right) (dG/dt) \] (3)

\[ H(t) = G(t) - \left( \frac{1}{\beta} \right) (dH/dt) \] (4)

and

\[ R(t) = A \sqrt{H(t)} + G(0) - R(0) \] (5)

where

- \( F(t) \) = Aortic root tyramine concentration time function
- \( B \) = An activity coefficient of tyramine
- \( H(t) \) = A time function defined by the first two equations
and \( 1/\beta \) = The natural time constant of the second unit-lag function.

A computer circuit (fig. 3B) similar to that used for norepinephrine simulation was used for tyramine effect simulation. A second series unit-lag circuit with a long time constant was necessary for satisfactory simulation. An activity coefficient was introduced to relate tyramine-effect relation to that of norepinephrine. As would be expected this coefficient was much attenuated in reserpinized preparations.

The equations describing the simulating circuitry are:
Figure 5 shows the correspondence between biologic and computer simulated responses for the normal and reserpinized case. Attenuation on B in the reserpinized case is more striking than one might assume by simply noting the relative rate increase compared to normal but this is explained by the relative "suprasensitivity" of the reserpinized preparation to norepinephrine.

Constraints on a System Model
An analog of the system response to norepinephrine and tyramine must simulate the experimentally derived information in all its important aspects. Certain hypotheses may be introduced and their value judged by their uniqueness or lack thereof in producing a certain transform, the likelihood of such a proposed mechanism in a biologic system and finally their potential for suggesting discriminating experimental designs.

The linear* and nonlinear elements of the system rate response as covered in the preceding sections must be reasonably approximated by the system model. The mass-response characteristics, in terms of norepinephrine in the coronary sinus efflux, must be considered. Thus, approximately 50% of norepinephrine entering the coronary arteries appear in the coronary sinus and a very small concentration of norepinephrine appears in the efflux after tyramine administration. The observed "interaction" between unbound norepinephrine and tyramine must be explained preferably without invoking a strategic liberation hypothesis.

Mathematical Considerations
If the modalities and magnitudes of the rates of exchange of a substance among spaces can be numerically expressed, the amounts of that substance in the spaces as a function of time are solutions of the simultaneous differential equations resulting from the exchange rate expressions. As the system increases in complexity, hand solution becomes difficult, protracted, and often impossible. In such systems the use of electronic analog computer circuitry to simulate the system may make solution rapid and facile and allow repeat display of the resultant of variation on problem parameters. This section will set forth the rate expressions describing the proposed exchange system.

The exchange of norepinephrine will be described among three spaces in series: 1) intravascular space, 2) receptor site space, 3) storage space.

The rates of change of mass in the intravascular space (V) are attributable to three factors:

1) Norepinephrine entering in the inflow blood equal to the concentration-time function (CTF) of the amine in blood entering the intravascular space multiplied by the blood flow:

$$\frac{\partial V}{\partial t} = CTF \times \text{Flow} \quad (6)$$

2) Norepinephrine leaving in the outflow

\[ a \]

\[ \frac{a}{s+a} \]

\[ \frac{B a f}{s^2 + (a+\beta)s + a\beta} \]

\[ \frac{s+a}{s+a} \]

\[ \frac{B a f}{s^2 + (a+\beta)s + a\beta} \]

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* In terms of Laplacian transfer function notation the general system model must approximate the following transforms for the linear part of the system; i.e., up to but excluding the nonlinear calibration transform:

For epinephrine: \[ \frac{a}{s+a} \]

For tyramine: \[ \frac{B a f}{s^2 + (a+\beta)s + a\beta} \]
blood. This was programmed as a single mixed dilutional volume with the rate of elution directly proportional to the flow and inversely related to the volume

$$\frac{dV}{dt} = V \times \text{Flow/Volume}$$  \hspace{1cm} (7)

3) Norepinephrine exchanging with receptor site space. This is programmed as a system with a partition coefficient greatly in favor of mass transfer to receptor site space. RS is the mass of norepinephrine in receptor site space.

$$\frac{dV}{dt} = -K(V - P \times RS)$$  \hspace{1cm} (8)

where $P$ is a small number and $K$ is the rate constant.

The total derivative of $V$ may now be expressed assuming there are no other significant pathways of norepinephrine in the vascular space:

$$RS(t) = RS(0) + \int_{0}^{t} K(V - P \times RS) - K'(Se)(RS) + K''(So) dt$$  \hspace{1cm} (14)

The rates of change of norepinephrine mass in the receptor site space $(RS)$ are attributed to two factors:

1) Norepinephrine exchange with intravascular space. Except for a change in sign this is identical with the third partial derivative discussed in reference to intravascular space.

$$\frac{dRS}{dt} = K'(Se)(RS)$$  \hspace{1cm} (11)

2) Norepinephrine taken up by storage sites. This is treated as a reversible reaction of the type $A + B \rightleftharpoons AB$ with equilibrium greatly in favor of formation of $AB$. This is second order from left to right and first order in reverse. Defining total storage site mass as $S_{max}$, those occupied by norepinephrine as $So$ and those unoccupied as $Se$; clearly $S_{max}$ equals $So$ plus $Se$.

$$\frac{dRS}{dt} = -K'(Se)(RS) + K''(So)$$  \hspace{1cm} (12)

The total derivative of $RS$ can be expressed:

$$dRS/dt = K(V - P \times EC) - K'(Se)(RS) + K''(So)$$  \hspace{1cm} (13)

and the mass of norepinephrine in the receptor site space at time $t$ when the mass at time zero is $RS(0)$ is given by the expression:

$$RS(t) = RS(0) + \int_{0}^{t} K(V - P \times RS) - K'(Se)(RS) + K''(So) dt$$  \hspace{1cm} (14)

The total derivative of stored norepinephrine, $So$, is given by:

$$dSo/dt = K'(Se)(EC) - K''(So)$$  \hspace{1cm} (15)

and the mass of norepinephrine stored at time $t$ when the mass stored at time zero is $So(0)$ is given by the expression:

$$So(t) = So(0) + \int_{0}^{t} K'(Se)(RS) - K''(So) dt$$  \hspace{1cm} (16)

The drug tyramine is considered to be in competition with norepinephrine for free storage sites in a manner akin to the kinetics of norepinephrine storage. Designating tyramine activity as $Ty$ and tyramine filled storage sites as $St$, we have for the derivative of tyramine storage:

$$dSt/dt = K'(Se)(Ty) - K''(St)$$  \hspace{1cm} (17)

and the integral equation is of identical form as for norepinephrine.

The storage space equality now becomes:

$$S_{max} = Se + So + St$$  \hspace{1cm} (18)

Note that tyramine is considered to be in

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Analog computer circuit for simultaneous solution of equations 6 through 18. For norepinephrine depletion (reserpinized) simulation base line rate automatically approximates +1 and for normal simulation +20 beats per minute. Appropriately scaled RS is concentration in the receptor site space and is used as the input to the calibration curve circuit. This circuit and that describing tyramine storage kinetics are omitted from the diagram for simplicity. CTF represents the concentration time curve of norepinephrine input. Diagram notation is consistent with that of the equations in the text with the exception of certain gain designations. QSM represents a quarter-square-multiplier in the multiplication mode. Except where noted otherwise, gain is unity.

Parameters:
- \( G = 0.0833 \)
- \( F/V = 0.833 \)
- \( K = 0.0833 \)
- \( P = 0.06 \)
- \( S_e = 40 \) (normal), \( 42 \) (reserpine)
- \( S_m = 80 \) (normal), 42 (reserpine)
- \( c = 0.023 \), \( a = b = 44 \), \( K' = 0.005 \), \( K'' = 0.005 \).

competition for storage with norepinephrine but not for preferential accumulation in the receptor site space.

**RESPONSE OF THE COMPUTER MODEL**

A computer circuit solving the equations for norepinephrine transfer is shown in figure 6. By proper selection of system parameters rather good approximations of the drug input, rate output transformation can be achieved (fig. 7). Such a system has excellent conformity to the mass input-output relationship in allowing the appearance of approximately half of infused norepinephrine at the "coronary sinus" and yet predicting the appearance of only small concentrations of tyramine released norepinephrine at that site even when tyramine is exerting a sizeable rate increase in a "preparation" with a normal complement of norepinephrine (fig. 8). No strategic release pathway is necessary to explain the discrepancy between tyramine effect and the small amount of norepinephrine released. Further, such a system will demonstrate an "interaction" between tyramine and unbound norepinephrine. By decreasing the rate of storage of infused norepinephrine, tyramine may be seen to have a potentiating effect; an action that is not attributable to release of norepinephrine from storage sites but that is in fact a corollary of the proposed mode of tyramine release of norepinephrine. A simulation of such interaction is seen in figure 9.

**Discussion**

The analysis of a system by analog simulation implies that a model is sought that corresponds in certain modalities to the real system behavior, given similar input pulses and/or initial states. The dynamic similarity of input-output relations of the real system and the model is no guarantee that the simulation is unique or that any component operational units bear a one-to-one correspondence to mechanisms in the real system. Depending upon a knowledge of the mechanics of the real system, the model may formally analog certain known operational units. Complementary mechanisms may then be proposed, always under the constraint that the overall model conform to the real system behavior in all important respects. This may lead to unifying hypotheses and suggest the design of experimental approaches to the real system. Paradoxically, a model whose components are incompletely identified with objective correlates in the real system is the most useful; when all the mechanisms of the real system are delineated the model reduces to an instrument of pedagogy. Models of the norepinephrine-tyramine interaction do not seem to be in present danger of this latter fate.

Nonlinear responses of biologic systems to pharmacologic agents are common phenomena. Many of these responses demonstrate successively decreasing increments of response for linear increments of drug in the range of drug dosage of interest to the investigator. Thus, the pharmacologic literature is replete with instances of "logarithmic" dose-response relationships. In certain instances the pharmacologic agent is present normally in the biologic system and maintains a base line level of response. In such systems, the simple depletion of endogenous drug should produce certain
Comparison of the response of the system model to the experimentally derived input-output transfer function for norepinephrine and tyramine in simulated normal (N) and reserpinized (R) preparations. Curve pair A is generated from an idealized norepinephrine input according to equations 1 and 2. Curve pair C is generated from the identical input by the system model (fig. 5). An excellent conformity of response is evident. Curve pair B is generated from an idealized tyramine input according to equations 3, 4, and 5. Curve pair D is generated from the system model in accordance with the equations describing tyramine competition for norepinephrine storage sites. The system model tends to underpredict the magnitude of tyramine effect and yield somewhat later peak effects but is generally in good conformity with the overall duration and magnitude of tyramine effect.

Parameters of the simulating systems: $a = 0.111$, $b = 0.0089$, $B(N) = 0.104$, $B(R) = 0.008$, $S_j(N) = 40$, $S_j(R) = 2$, otherwise as in figure 5.

Predictable results. The base line level of response should be lessened if depletion is of sufficient magnitude. The response to a given initial increment of administered drug should be increased as compared with the undepleted because it operates over a portion of the dose-response curve having a steeper slope. The normal response curve should be in close conformity to the upper portion of the curve equal peak responses. Solid line represents the residual exogenous norepinephrine appearing in the coronary sinus blood after an injection of that drug. Dotted line represents the slower and smaller concentrations appearing as the result of the release of endogenous norepinephrine by tyramine.

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from the more sensitive, depleted preparations. Within the limits of biological variability, the norepinephrine chronotropic effects in the canine heart-lung preparation demonstrate these attributes. The response is clearly nonlinear, initial rates are lower in depleted preparations which are "suprasensitive," and finally the normal response and the upper portion of the depleted response are similar. No mechanism for the nonlinearity itself is proposed in the present formulation. The relation is simply approximated by a satisfactory fitting and easily generated continuous function. It is suggested, however, that no separate mechanistic hypothesis need be proposed to explain depletion suprasensitivity but that the phenomenon may be viewed as a corollary of the system response nonlinearity.

Depletion of endogenous norepinephrine is not the only possible mechanism that might increase sensitivity to exogenous amine. Mechanisms that might greatly decrease the rate of storage of norepinephrine would bring about suprasensitivity, e.g., reduction of free storage sites or interference with the unknown mechanisms that bring about this storage. Suprasensitivity might then appear without a significant alteration in the amount of stored norepinephrine. Similar mechanisms have been proposed as a possible explanation for suprasensitivity without storage depletion in the decentralized nictitating membrane of the cat.8 In the canine heart-lung preparation tyramine rate responses and inotropic effects are consistently later in reaching maximal values and of much longer duration as compared to responses to norepinephrine.8 Aspects of this temporal discrimination have been noticed by many investigators but quantitative description of the time course of these responses has not heretofore been set forth. Such analysis requires a definition of the temporal course

Allowed in the real experiment, was not simulated.

Curve pair C illustrates the proposed mechanism for tyramine "interaction" with unbound norepinephrine. Values on the abscissa represent norepinephrine entering the system. Ultimately total storage is approximately the same but the relatively slower rate of storage in the presence of tyramine would explain the greater and more prolonged effects of the infused norepinephrine.
of drug entering the system and that its presentation and removal be relatively rapid compared to the time course of response. These conditions are most readily fulfilled in perfused preparations. From the nature of the transfer function for tyramine it is clear that the prolonged response to this drug is not simply attributable to the recirculation of unmetabolized drug. With the exclusion of recirculation, tyramine still has a much longer time course of response than norepinephrine. The release of norepinephrine from the heart by tyramine is slow and the increase in coronary sinus concentration is small. Based upon a bio-assay, the response of aortic spiral strips to bath fluid in which normal atria were exposed to tyramine, Hall concluded that either a large amount of the catechol amine liberated by tyramine is destroyed or that a much smaller amount of endogenous material than of added norepinephrine is required to produce the same effect. This prolonged release of relatively small amounts of norepinephrine by tyramine while producing a sizeable biologic response of long duration suggests that the response characteristic might be the result of a capacitative mechanism for norepinephrine in the receptor site space. This proposal taken with the assumption that tyramine competes for storage sites with norepinephrine forms the major hypothetical formulation upon which the system model is based. The latter assumption is clearly a non-unique kinetic solution; reduction in the rate of norepinephrine storage by interference with the processes of storage (reduction of $K'$ in the model) would be equivalent to the reduction of available storage sites ($S_e$). While the former possibility would not have a predictable rate limitation the latter mechanism would be at least rate limited by the time course of tyramine available for storage competition. In the latter case one would predict a significant amount of tyramine to be incorporated into storage sites. In isolated chromaffin granules the amount of released catechol amine under the influence of tyramine is replaced stoichiometrically by an uptake of tyramine. In the rat heart, perfusion with tyramine may result in a greater concentration of that amine than of norepinephrine originally present. In addition, norepinephrine inactivation by catechol-O-methyl transferase, the major pathway of inactivation of tyramine released norepinephrine, is not inhibited by tyramine although its ability to metabolize the excess norepinephrine liberated appears to be limited. Thus, the assumption of competition between the amines for storage sites is supported by some direct evidence and there appears to be no significant norepinephrine “potentiation” by the suppression of normal metabolic pathways of inactivation of tyramine. Since the amounts of tyramine stored in the heart are of sufficient magnitude to be consistent with the concept of competitive storage, one would predict that a certain portion of the stored tyramine would be released by increasing the norepinephrine content of the organ perfusate. Further investigation on this point is clearly indicated. In addition, the study of the alteration by other agents of the response to norepinephrine and tyramine-like drugs, with regard to temporal aspects, may prove more profitable in defining the mechanism of alteration than the mere recording of peak responses.

A kinetic model based upon these hypotheses will demonstrate “interaction” between tyramine and unbound norepinephrine. Smith concludes that since the action of tyramine may be enhanced in the presence of increased extracellular norepinephrine, its action may not be explained solely by an ability to release norepinephrine from tissue stores. Viewed in the dynamic aspects presented herein which reject the connotative confines of the term “release” in favor of a mechanistic description, what is considered to be the “normal” mode of tyramine action and the “interaction” of tyramine and unbound norepinephrine are two aspects of the same phenomenon. What is apparently an evocative effect in the normal mode becomes a permissive effect when tyramine decreases the rate of norepinephrine storage by occupying storage sites. This is most readily observed in the absence of stored norepinephrine. Simulation of the permissive effect is demonstrated in figure 9.
Storage space inhomogeneity as first proposed by Trendelenburg is not simulated in this model. Different half-lives have been demonstrated for exogenous norepinephrine entering a smaller tyramine-releasable pool and that entering a larger tyramine-resistant pool with a slow transfer from the former to the latter. Since the events simulated in the present formulation are relatively rapid the storage space simulated may be assumed to be the available or tyramine releasable portion. The capacity of the storage space cannot be assigned a meaningful numerical value as the values that enter the simulation are rate constant-storage size products and cannot be separately evaluated.

Presently, extrapolation from the chronotropic effects of the amines considered here to other modalities of their biologic response must be cautious. The present study does suggest, however, that a system response analysis might be fruitful in a system in which response modality is a simply dependent variable and can be continuously quantified.

Summary

An analog simulation of the chronotropic effects of norepinephrine and tyramine in the canine heart-lung preparation is presented. The temporal aspects of those responses are described in quantitative terms.

Reserpine produced deprivation supersensitivity to norepinephrine may be considered as a corollary of the system response nonlinearity to that drug.

Discrepancies between tyramine rate responses and the relatively small amounts of norepinephrine released are resolved by the assumption that the receptor space for norepinephrine has a capacitative function and is not in free dilutional equilibrium with the perfusing blood. This mechanism would allow for a unitary explanation of tyramine action based upon competition with norepinephrine for available storage sites.

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