A PROPOSED KINETIC MECHANISM FOR ENZYMIC REGULATION OF THE BLOOD PRESSURE, BASED ON CLINICAL EXPERIENCE

RENÉ DYBKÆR

Central Laboratory at Frederiksberg Hospital and
Physico-Chemical Institute of the University of Copenhagen (Denmark)

The human blood pressure is controlled by a combination of nervous and hormonal factors, and normally this regulation is very sensitive and efficient.

Noradrenalin plays a double role in this regulation; it is the humoral transmitter of the sympathetic nervous impulses to the muscular walls in the vessels (the end organ), and—in addition to adrenalin—it is a circulating pressor hormone produced in the chromaffin cells of the adrenal medulla.

The elimination of these very potent pressor amines is rapid and their total concentration is normally below 2 μg per liter blood (LUND[1]).

A *phaeochromocytoma* is a benign tumor in the hormone-producing chromaffin tissue and the richly varied symptomatology of this disease is explained by a continuous or intermittent secretion of pressor amines, noradrenalin usually predominating.

The immediate response to a sudden augmentation in circulating pressor hormones is hypertonia. This will stimulate the pressor zones causing decreased secretion of noradrenalin from the nerve endings. The nervous counter-measures following a sudden "auto-injection" of pressor substances will probably inhibit the hypertensive reaction. With greater amounts of pressor amines, however, an increase in the elimination of pressor hormones is necessary, if observed clinical reactions are to be explained.

Renal excretion of pressor substances is one way of elimination, but this is insufficient—especially with increased production of hormones (GOLDENBERG, SERLIN, EDWARDS AND RAPPORT[2]). It is reasonable, then, to examine whether a more rapid *enzymic elimination* of adrenalin and noradrenalin may influence the blood pressure in patients with abnormally high production of pressor hormones.

The following kinetic mechanism will be found to explain the facts of different patterns of blood pressure variations, seen in patients with phaeochromocytoma.

THE HYPOTHETICAL NORMAL REACTION MECHANISM AND ITS KINETICS

Pressor amines (A) from nerve-endings or adrenal medulla are known to be oxidatively deaminated by the enzyme amine oxidase (E), giving a product (A') without effect on the blood pressure. The degradation product is probably eliminated through a series of reactions and at least one of these is irreversible.

It is very likely that the enzymic change of A to A' is achieved via an enzyme-substrate complex (EA), as originally claimed by MICHAELIS AND MENTEN[3]. The

References p. 481.

complex is cleaved into A′ and free enzyme (E), or possibly another complex Ex, eventually giving free enzyme. In this way, the catalytic nature of the enzyme is established.

Schematically, the sequence of reactions described is:

$$\text{hormone production} \rightarrow A \tag{1}$$

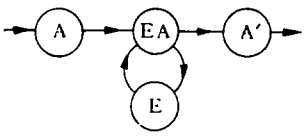
$$A + E \rightleftharpoons EA \tag{2}$$

$$EA \rightleftharpoons Ex + A' \tag{3}$$

$$A' \rightarrow \text{irreversible elimination} \tag{4}$$

$$Ex \rightleftharpoons (\text{other complexes?}) \rightleftharpoons E + x \tag{5}$$

As one step at least is irreversible (4) the length of the chain is of minor importance, and A is continuously inactivated to A′ following scheme I.



Scheme I

Another set of reactions, however, must be considered. The synthesis of free enzyme may be via a proenzyme, and the stepwise degradation with at least one irreversible reaction.

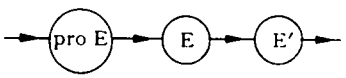
$$\text{Synthesis} \rightleftharpoons \text{proenzyme} \tag{6}$$

$$\text{proE} \rightleftharpoons E \tag{7}$$

$$E \rightleftharpoons E' \tag{8}$$

$$E' \rightarrow \text{irreversible degradation} \tag{9}$$

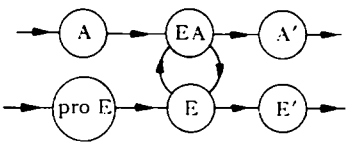
From our point of view the important reactions are



Scheme II

and the irreversible step will ensure a continuous degradation of E.

The two schemes may of course be combined in one.



Scheme III

All the reactions given are assumed to follow the usual kinetic rules and are characterized by rate constants, dependent on temperature. The reaction rate of any single process in one direction will depend on the rate constant in this direction and the concentrations of reactants.

With several reversible steps the problem would be very complicated. The occurrence of irreversible steps, however, simplifies the treatment and ensures a continuous "flow" towards elimination, and it can be shown that in any case the system will tend to attain a *steady state*. In the ideal steady state the concentration of each reacting substance is constant and the relative values of concentrations are adjusted to the rate constants in such a way that *all* reaction rates in the sequence are equal.

References p. 481.

With respect to the enzyme the steady state is achieved practically momentaneously as its concentration probably is small compared to the amount of hormone (BRIGGS AND HALDANE[4]).

In a closed system, the ideal steady state– in which the concentration of the hormone is also constant- cannot be maintained before equilibrium has been attained. In our case, however, the addition of A at a constant rate, the reproduction of E (scheme I) and the presence of an irreversible step conserve the steady state as long as no other changes are made.

The production and elimination of enzyme (scheme II) has not yet been considered, but in the steady state this system will cause no deviations from the principles outlined since production and destruction of enzyme will be equal. It is furthermore reasonable to suppose that the rate of the inactivation of pressor hormone is relatively large, whereas enzyme synthesis and destruction are relatively slow processes.

It should be emphasized that when the production of A is unchanged the adjustment and maintainance of a steady state is the inevitable consequence of any reaction sequence with an irreversible step.

In other words: The circular process in scheme I will "rotate" with a rate sufficient to inactivate exactly the amount of pressor substance produced. A slight destruction of enzyme is balanced by synthesis.

Thus the *blood pressure* is maintained at a constant (normal) level if all other conditions be unchanged.

How the postulated system will react to abnormal and changing hormone concentrations, as seen in patients with phaeochromocytoma, will now be discussed.

CONSTANTLY INCREASED PRODUCTION OF PRESSOR SUBSTANCES

When the concentration of A ('concentration of A' will be symbolized by [A]; this also applies to other substances) increases, the rate of reaction (2) to the right will instantaneously augment, resulting in a higher [EA] which causes an increase in the rate of (3) to the right. The circular process in scheme I "rotates" with increasing velocity, thus inhibiting the rise in [A]. With [A] increasing the rate of inactivation will likewise increase, but more and more slowly; finally, the inactivation of A compensates the production. A new steady state is then attained with a higher hormone concentration and, consequently, a higher blood pressure, as seen in many patients with phaeochromocytoma and constant hypertension.

It should be noted that in the new state a greater part of total enzyme than normal is bound in complexes, indeed this is necessary to obtain a higher rate of destruction of A. The decrease in [E] elicits an increased production of enzyme according to (7) and (6) and a decreased inactivation via (8) and (9); thus [E] again approaches normal values.

The rates of the processes to the right in scheme II are supposed to be small and some time elapses before a considerable production of enzyme ensues; it will reach significant amounts eventually if a high [A] is maintained, and the enzyme produced is "divided" between E and EA in the right-shifted reaction (2) until [E] is normal.

The following example may illustrate the two steady states described.

References p. 481.

For reaction (2) we have in the steady state

$$[A]\,[E] = K\,[EA] \tag{10}$$

where K is the Michaelis constant (which is not identical with the equilibrium constant). If we put

$$[E] + [EA] = [E_{tot}] \quad \text{or} \quad [E] = [E_{tot}] - [EA]$$

equation (10) becomes

$$[A]\,([E_{tot}] - [EA]) = K\,[EA] \quad \text{or} \quad [EA]\,(K + [A]) = [A]\,[E_{tot}]$$

which is the same as

$$[EA] = \frac{[A]\,[E_{tot}]}{K + [A]} \tag{11}$$

Combining (10) with (11) we get

$$[E] = \frac{K\,[E_{tot}]}{K + [A]} \tag{12}$$

Let us examine the normal steady state (I), with a normal, constant hormone concentration. If $[E_{tot}]_I = 1$, $[E]_I = 10\,[EA]_I$ and, say $[A]_I = 10$, then from (10) we get $K = 100$, and (11) and (12) give

$$[EA]_I = \frac{10 \times 1}{100 + 10} = \frac{0.1}{1.1} \quad \text{and} \tag{13}$$

$$[E]_I = \frac{100 \times 1}{100 + 10} = \frac{1.0}{1.1} \tag{14}$$

In some patients with phaeochromocytoma the hormone concentration may reach values twenty times the normal (VON EULER[5]). In the corresponding semi-steady state (II)—before the total enzyme concentration is appreciably augmented—the equations (11) and (12) give

$$[EA]_{II} = \frac{200 \times 1}{100 + 200} = \frac{2}{3} \quad \text{and}$$

$$[E]_{II} = \frac{100 \times 1}{100 + 200} = \frac{1}{3}$$

With time the concentration of free enzyme becomes normal (III), *i.e.*

$$[E]_{III} = [E]_I = \frac{1.0}{1.1}$$

and from (10)

$$\frac{200 \times 1}{1.1} = 100\,[EA]_{III}$$

$$[EA]_{III} = \frac{2.0}{1.1} \quad \text{and then}$$

$$[E_{tot}]_{III} = \frac{3.0}{1.1}$$

As the concentration of total enzyme in the normal steady state was unity it will be seen that a twenty-fold increase in substrate concentration has increased the concentration of total enzyme nearly three times. Nevertheless, the rate of inactivation of hormone—following the first order reaction (3)—is increased twenty times as the concentration of EA has been increased twenty times relative to normal values.

References p. 481.

SUDDEN FALL IN PRODUCTION OF PRESSOR HORMONES FOLLOWING AN INCREASE OF LONGER DURATION

We assume that a steady state (III) exists at a high pressor level and with increased total enzyme concentration in the circular process (scheme I).

A sudden decrease in the *production* of A to normal or subnormal levels will cause a decrease in the rate of elimination (process I) which is determined by [A]. At the same time [EA] must fall and [E] consequently increase.

The moment (IV) when [A] reaches normal values, [E] and [EA] are both approximately three times higher than normal.

Continuing with the example we have

$$[EA]_{IV} = \frac{10(3/1.1)}{100 + 10} = \frac{0.3}{1.21}$$

$$[E]_{IV} = \frac{100(3/1.1)}{100 + 10} = \frac{3}{1.21}$$

The higher concentration of EA than under normal conditions means a higher rate of destruction of hormone and as the hormone *production* is normal, the hormone *concentration* becomes subnormal in the end organ; a new semi-stationary state (V) is attained with subnormal blood pressure and perhaps clinical shock.

In this state the rate of hormone inactivation is normal, *i.e.* (see (13))

$$[EA]_V = [EA]_I = \frac{0.1}{1.1} \quad \text{and as}$$

$$[E_{tot}]_V = [E_{tot}]_{III} = \frac{3.0}{1.1}$$

$$[E]_V = \frac{3}{1.1} - \frac{0.1}{1.1} = \frac{2.9}{1.1}$$

From (10) we get

$$[A]_V = \frac{100 \cdot \frac{0.1}{1.1}}{\frac{2.9}{1.1}} = \frac{10}{2.9} \sim \frac{1}{3}[A]_I$$

The concentration of active pressor hormone is thus decreased to about one third of normal values, and the blood pressure must consequently fall below normal levels.

The clinical condition described is often encountered after the operative removal of phaeochromocytoma.

As mentioned above the concentration of E (in V) is still above normal and consequently the enzyme production decreases (7), and as enzyme destruction is increased ((8)–(9)) a steady state is reached eventually with a normal hormone concentration and a normal blood pressure. In some cases, however, the state of shock is severe and the patient succumbs if immediate treatment with noradrenalin is not instituted.

That readjustment to a normal steady state may be a rather slow process is substantiated by the fact that post-operative pressor treatment is sometimes required for hours or days.

Analogously the discontinuance of noradrenalin therapy should be gradual when

References p. 481.

a patient has been treated with larger doses of pressor hormone for a prolonged period.

In some cases hypertonia persisted despite operation (GOLDENBERG, ARANOW, SMITH AND FABER[6]). These patients had possibly developed irreversible organic damages of the renal vessels consequent to a hormonal hypertonia and thus suffered from an acquired renal hypertension.

The blood pressure response to a certain amount of noradrenalin or adrenalin is less in patients with phaeochromocytoma and constant hypertension than in normal persons and in patients with essential hypertension (GOLDENBERG *et al.*[6]).

The observed decrease in sensitivity is possibly identical with the increase in enzyme concentration and is naturally explained by the reaction scheme: A certain amount of hormone is inactivated more rapidly at a high enzyme concentration, when the rate of inactivation is great, than at normal enzyme level with a slower conversion rate. The organism will react relatively, rather than absolutely (Weber's law).

MODERATE HYPERTENSIVE CRISES OF SHORTER DURATION

Such events present no new problems as long as the production of hormone is augmenting. The reactions will shift to the right until destruction balances production.

It is assumed that shorter increases in hormone level do not appreciably influence the enzyme production.

This is explained by small rate constants in scheme II. When hormone production is again normalized the steady state also reverts to a normal rate and as $\bar{E}$ becomes normal at the same time, no transitional phase occurs with subnormal hormone concentration in the end organ.

The sequence is in agreement with clinical experience. Shorter and less severe hypertensive crises in otherwise healthy patients with phaeochromocytoma are not followed by shock.

CONCLUDING REMARKS

The reaction mechanisms and the kinetics presented are hypothetical; they are however rather simple, plausible and furthermore agree with and explain several clinical facts in the manifold picture of the phaeochromocytoma.

Proof of the kinetics will require perfusion-experiments with isolated vessels and measurement of variations in hormone and enzyme concentrations with time.

The presentation of a hypothetical kinetic mechanism is perhaps—until then—justified by the words of CHRISTIANSEN[7]: "With reaction kinetics one is forced to postulate a mechanism which explains the empirically found kinetics. It is impossible, a priori, to choose the mechanism, as there are always several possibilities and only the kinetics can prove which one is correct".

Coupled flow-reactions of substrate inactivation and enzyme production and destruction (Scheme III) may well help to explain other physiological and pathological mechanisms. One example which is analogous to the phaeochromocytoma is the disease called carcinoid (see *e.g.* JENSEN[8]), where varying amounts of 5-hydroxytryptamine are injected in the blood stream from the secreting chromaffin tumour-tissue in the alimentary tract. This amine is also inactivated by a specific amine oxidase in the liver and lung.

References p. 481.

ACKNOWLEDGMENTS

The author's thanks are due to Professor J. A. CHRISTIANSEN and A. LEVIN NIELSEN for much helpful criticism.

SUMMARY

An explanation is sought for the normal stability of the blood pressure and the variations found in different forms of phaeochromocytoma. A rather simple scheme of reactions involving enzymic degradation of pressor amines and another scheme for production and inactivation of the deaminating enzyme is proposed. By applying the principle of the steady state to the combination of these reactions many clinical facts are explained. Quite similar reaction mechanisms may help to explain other physiological and pathophysiological phenomena.

REFERENCES

1 A. LUND, *Scand. J. Clin. & Lab. Invest.*, 4 (1952) 263, and personal communications (1956).
2 M. GOLDENBERG, I. SERLIN, T. EDWARDS AND M. M. RAPPORT, *Am. J. Med.*, 16 (1954) 310.
3 L. MICHAELIS AND M. L. MENTEN, *Biochem. Z.*, 49 (1913) 333.
4 G. E. BRIGGS AND J. B. S. HALDANE, *Biochem. J.*, 19 (1925) 338.
5 U. S. VON EULER, *Scand. J. Clin. & Lab. Invest.*, 5 (1953) 122.
6 M. GOLDENBERG, H. ARANOW, A. A. SMITH AND M. FABER, *Arch. Internal Med.*, 86 (1950) 823.
7 J. A. CHRISTIANSEN, *Acta Chem. Scand.*, 8 (1954) 909; *Fysisk Tids.*, 53 (1955) 200.
8 K. JENSEN, *Danish Med. Bull.*, 4 (1957) 96.

Received April 2nd, 1957

INFLUENCE DU TAUX DE CROISSANCE SUR LA CONSTITUTION DU SPECTRE HÉMATINIQUE DE *B. SUBTILIS*

P. CHAIX ET J. F. PETIT

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (France)

Dans deux précédentes publications[1,2], il a été montré que le spectre de *B. subtilis* cultivé sur bouillon de viande peptoné et gélosé présente d'importantes variations suivant que les bactéries sont récoltées après des temps d'incubation plus ou moins longs. Dans le cas de cultures de longue durée, l'examen spectrographique révèle, à la température ordinaire et à $-190°$, les trois composantes cytochromiques classiques *aa*, *ba* et *ca*, tandis que, dans le cas de cultures de courte durée, cet examen révèle à la température ordinaire les bandes *aa* et b_1a, cette dernière se scindant à $-190°$ en 4 bandes: *ba* (561 mμ), *ya* (556–557 mμ), *za* (553–554 mμ) et *ca* (548–549 mμ).

Ces observations avaient été faites sur des microorganismes s'étant développés dans des conditions difficiles à analyser. Il était donc intéressant de reprendre l'étude du phénomène sur des bactéries obtenues dans des conditions simples, rigoureusement définies.

Pour ce faire nous avons réalisé des cultures de *B. subtilis* sur milieux liquides synthétiques agités en présence d'air dans des conditions telles que l'oxygène ne soit pas facteur limitant de la croissance pendant la durée de l'expérience, et nous avons fait varier la nature du substrat carboné. Les bactéries étant récoltées au cours de

Bibliographie p. 486.