A Model for the Passage of the Neurohypophysial Hormones and their Related Proteins through the Rat Neurohypophysis

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The change in the radioactivity of vasopressin–neurophysin in the rat neurohypophysis after an intracisternal injection of [^{35}S]cysteine was fitted to several mathematical models. The data fitted best a model in which there is a linear input of radioactive protein into one pool of the neurohypophysis, from which it is either released by an exponential process or transferred to a second pool from which it is released by a second exponential process with a rate constant much lower than the first. This model is compatible with the existence of a ‘readily releasable’ pool first postulated by Sachs *et al.* (1967). Data for the change in radioactivity of vasopressin also gave a good fit in this model. Calculation of the rate constants suggested that the first pool represented about 2% of the total hormone.

When the neural lobe of the pituitary gland is stimulated to release its hormones, either *in vivo* (Sachs *et al.*, 1967) or *in vitro* (Thorn, 1966; Sachs & Haller, 1968) there is an initial rapid increase in the rate of hormone release, but this is not sustained with continued stimulus even though only a very small proportion of the total gland content of hormone has been released. If the stimulus is removed, a second stimulus does not elicit any increase in the rate of release unless a considerable time is allowed to elapse between the two stimuli. These observations led Sachs *et al.* (1967) to propose that the hormone was not homogeneously distributed in the gland but that there were at least two pools: a ‘readily releasable’ pool from which hormone could be rapidly discharged and a second pool from which it was released more slowly. It would appear (Sachs & Haller, 1968; Sachs, 1971) that newly synthesized hormone enters the ‘readily releasable’ pool before it equilibrates with the larger storage pool, because when glands labelled *in vivo* were stimulated they released hormone with a higher specific radioactivity than that remaining in the gland.

In our study of the turnover of the neurohypophysial hormones (Jones & Pickering, 1972) and of their related proteins, the neurophysins (Burford & Pickering, 1973), we found evidence that new material disappears from the gland at an exponentially decreasing rate. This would be expected if there were a single homogeneous pool in the gland. One can reconcile these findings with the presence of a ‘readily releasable’ pool if it is assumed that this pool is only important during a relatively severe pharmacological stimulus and does not play any part in the normal physiological release mechanisms. On the other hand, another inter-

pretation might be that the neurosecretory products spend only a relatively short time in the ‘readily releasable’ pool, so that the contribution of this pool to the overall disappearance kinetics studied during a 5-week period would be small. The apparent exponential disappearance of radioactive neurophysin from the gland (Burford & Pickering, 1973) would then arise because the small contribution of the ‘readily releasable’ pool at very early time-intervals would be swamped by the later events and be indistinguishable from error.

Because of the undoubted presence of a pharmacologically demonstrable ‘readily releasable’ pool (Sachs *et al.*, 1969; Sachs, 1971) we have examined our neurophysin data (Burford & Pickering, 1973) to see if they would better fit a mathematical model incorporating such a concept. It was thought that the hormone data, being derived from multiple bioassays, would not be precise enough for mathematical analysis but that the neurophysin results would not suffer from this disadvantage.

Methods

Data

The data for the appearance and disappearance of radioactive neurophysin in the neural lobe are from Burford & Pickering (1973). The neurophysin analysed was neurophysin-A, which is considered (Burford *et al.*, 1971) to be biosynthetically associated with vasopressin.

Derivation of the mathematical models tested

Basic assumptions. If we assume that the hypothalamo–neurohypophysial system is in equilibrium and that this equilibrium is not disturbed by the

introduction of a quantity of radioactive neurophysin, the amount of the radioactive material in the neural lobe at any given time will depend on the rates of input and output of such material. We suppose that, as a result of the initial injection at time $t = 0$, a total amount R of radioactive material will eventually pass through the neurohypophysis. Let $I(t)$ be the proportion of this material that will have reached the neural lobe by time t . We call $I(t)$ the input function. Similarly, for material newly arrived at the lobe we define $O(t)$, the output function, to be the proportion of such material which will have left after period t . If $P(t)$ is the proportion of R present in the lobe at time t then it will equal the input up to that time minus the reduction due to intermediate output $O(t)$:

$$P(t) = I(t) - \int_0^t O(t-x)I'(x)dx \quad (1)$$

where $I'(x)$ is the derivative of $I(x)$ and x is the integrating variable which ranges from zero to t . Thus $C(t)$, the amount of radioactive material present at time t , is $RP(t)$, or, substituting in eqn. (1):

$$C(t) = R\{I(t) - \int_0^t O(t-x)I'(x)dx\} \quad (2)$$

Our purpose is to compare the shape of the curve $C(t)$ with the empirical data by using various hypothetical input and output functions.

Input functions. We consider two types of input function. The first, which we denote by L , is input at a constant rate for a fixed time T . Such a model would result from saturation at some stage in the production process. In this case we have $I(t) = t/T$ for $t < T$ and $I(t) = 1$ for $t > T$, that is, a linear increase in $I(t)$ for a period T . The second model, E , presupposes the usual first-order kinetics for exit from a production pool, so that if the rate of release from the production pool is α we have $I(t) = 1 - \exp(-\alpha t)$, that is, input decreases exponentially.

Output functions. The general class of output functions we consider arises from a two-pool model shown in Scheme 1, and is based on the suggestions of Sachs and his colleagues (e.g. Sachs & Haller, 1968). Thus all input enters the first pool, from which it may escape either completely from the lobe at rate k_1 or by entering the second pool at rate k_2 . Escape from the second pool is then at rate k_3 . For this model we have:

$$1 - O(t) = \frac{k_1 - k_3}{k_1 + k_2 - k_3} e^{-(k_1 + k_2)t} + \frac{k_2}{k_1 + k_2 - k_3} e^{-k_3 t} \quad (3)$$

We denote this double exponential output by EE .

As a special case we can consider that entry to the second pool is impossible, $k_2 = 0$, that is the second pool does not exist; then we have a single exponential output, E , for which

$$1 - O(t) = e^{-k_1 t} \quad (4)$$

Substituting eqn. (3) and (4) into eqn. (2) we obtain:

$$C(E|EE) = R \left\{ \frac{\alpha(k_1 - k_3)}{(k_1 + k_2 - k_3)(\alpha - k_1 - k_2)} (e^{-(k_1 + k_2)t} - e^{-\alpha t}) + \frac{\alpha k_2}{(k_1 + k_2 - k_3)(\alpha - k_3)} (e^{-k_3 t} - e^{-\alpha t}) \right\} \quad (5)$$

$$C(L|EE) = R \left\{ \frac{k_1 - k_3}{(k_1 + k_2 - k_3)T(k_1 + k_2)} (1 - e^{-(k_1 + k_2)t}) + \frac{k_2}{(k_1 + k_2 - k_3)Tk_3} (1 - e^{-k_3 t}) \right\} \text{ for } t < T$$

$$= R \left\{ \frac{k_1 - k_3}{(k_1 + k_2 - k_3)T(k_1 + k_2)} e^{-(k_1 + k_2)t} (e^{(k_1 + k_2)T} - 1) + \frac{k_2}{(k_1 + k_2 - k_3)Tk_3} e^{-k_3 t} (e^{k_3 T} - 1) \right\} \text{ for } t > T \quad (6)$$

Scheme 1. Diagram representing the two-pool hypothesis for the passage of hormone through the neurohypophysis

and in the particular cases with $k_2 = 0$ we have:

$$C(E|E) = R \left\{ \frac{\alpha}{\alpha - k_1} (e^{-k_1 t} - e^{-\alpha t}) \right\} \quad (7)$$

$$C(L|E) = R \left\{ \frac{1}{Tk_1} (1 - e^{-k_1 t}) \right\} \text{ for } t < T$$

$$= R \left\{ \frac{1}{Tk_1} e^{-k_1 t} (1 - e^{-k_1 T}) \right\} \text{ for } t > T \quad (8)$$

Fitting the curves

Curves were fitted by an ICL475 computer by using Sampson's (1969) program (BMDX85) for non-linear least squares. In all computations, time zero was taken as 1 h after the intracisternal injection of [35 S]cysteine, because radioactive neurophysin does not begin to arrive in the gland until after this time (Burford & Pickering, 1973). The use of the BMDX85 program requires the calculation of derivatives of the hypothetical curves with respect to the unknown parameters. A reparametrization of the equations was made to give:

$$C(E|EE) = a_1(e^{-a_3 t} - e^{-a_4 t}) + a_2(e^{-a_5 t} - e^{-a_4 t}) \quad (9)$$

and

$$C(L|EE) = b_2(1 - e^{-b_4 t}) + b_1(1 - e^{-b_3 t}); t < T$$

$$= b_2(e^{-b_4(t-T)} - e^{-b_4 t}) + b_1(e^{-b_3(t-T)} - e^{-b_3 t}); t > T \quad (10)$$

The curves were fitted in the reparametrized form and the old parameters were then solved in the equations relating the old and new parameters. Thus for $C(L|EE)$ we have:

$$k_1 = (b_1 b_3^2 / b_2 + b_4^2) / (b_1 b_3 / b_2 + b_4)$$

$$k_2 = b_4 - k_1$$

$$k_3 = b_3$$

The BMD program is not suitable for the estimation of T in $C(L|EE)$. The estimate was obtained by the least-squares criterion but a trial-and-error procedure was adopted by using 0.5 h increments from $T = 10$ to $T = 13$ and determining the value which gave lowest total squared error; the results indicate that the solution is stable.

Results

Comparison of models

Fig. 1 shows the lines of best fit for the four different models studied (see under 'Methods') compared with the data (Burford & Pickering, 1973) for the passage of vasopressin-neurophysin through the neural lobe of the rat, and in Table 1 the models are compared in terms of the minimized total squared error of the observations from the fitted curves.

The total squared error within grouped data points was also computed to enable us to determine whether more complicated models could be justified. We see that the inclusion of a double-exponential output generally produces a significant improvement in fit ($P < 0.05$). Analysing the between-group variation not explained by the models, we find that there is a significant inadequacy of the model $E|EE$ ($P < 0.01$) whereas no significant improvement may be made over the fit of the model $L|EE$ ($P > 0.25$). Thus the lowest total mean square was obtained for the model (shown in Scheme 1) that describes a linear input to a pool in the gland from which there are two exponential units, one out of the gland and the other to a second pool from which there is another exponential exit from the gland. The curve plotted in Fig. 1(d) describes the situation when the linear input of radioactive neurophysins lasts from 1 h after injection until 12 h after injection ($T = 11$) as this value gave the lowest error of all the values tested within a certain range ($T = 10 - T = 13$). The values of the three rate constants (see Scheme 1) calculated from this model were: $k_1 = 2.32 \times 10^{-2} \text{ h}^{-1}$; $k_2 = 7.70 \times 10^{-2} \text{ h}^{-1}$; $k_3 = 1.38 \times 10^{-3} \text{ h}^{-1}$.

Jones & Pickering (1972) reported the change in specific radioactivity of the neurohypophysial hormones that occurs after an intracisternal injection of [^3H]tyrosine. Fig. 2 shows the computer-derived best fit for the vasopressin data to the model ($L|EE$) described by Scheme 1. The values for the rate constants were calculated to be: $k_1 = 0.46 \text{ h}^{-1}$; $k_2 = 11.23 \times 10^{-2} \text{ h}^{-1}$; $k_3 = 2.70 \times 10^{-3} \text{ h}^{-1}$. It must be noted, however, that we did not have any data for the crucial period between 12 h and 48 h after injection.

Discussion

After an intracisternal injection of [^{35}S]cysteine, the change in radioactivity of vasopressin-neurophysin in the neural lobe could be fitted best to an equation that describes the model shown in Scheme 1. Any attempt to simplify the model significantly increased the error in the resulting curve (Fig. 1). According to the model there is a continuous passage of the protein into the neural lobe and introduction of the tracer labels a 'slug' of protein, so that build-up of radioactivity in the neural lobe is linear while the 'slug' is entering the gland (i.e. for 12 h after the injection). The linearity of the build-up was apparent (Burford & Pickering, 1973) from simple plots of the data and suggests that free labelled amino acid is present in the hypothalamic secretory cells for a very short time relative to that required for assembly and transport of the neurohypophysial protein molecules.

On arrival in the neural lobe the model predicts that the protein first enters a pool from which it may be released through a process with a relatively

Fig. 1. Computer-derived curves of best fit for four models for the passage of radioactive neurophysin through the neurohypophysis of the rat

The models fitted described: (a), exponential input and single exponential output (E|E); (b), exponential input and two exponential outputs (E|EE); (c), linear input and a single exponential output (L|E); (d) linear input and two exponential outputs (L|EE). The experimental points are shown as means $\pm$ S.E.M. of 4–11 observations (see Burford & Pickering, 1973).

high ($2.32 \times 10^{-2} \text{ h}^{-1}$) rate constant or be transferred to a pool from which it is removed by a process with a much lower ($1.38 \times 10^{-3} \text{ h}^{-1}$) rate constant. It can be shown that the relative sizes of the pools is given by k_2/k_3 so that the first pool represents about 2% of the total gland content.

During their studies on hormone release Sachs and his colleagues (e.g., Sachs *et al.*, 1969) found that about 10% of the total hormone of the

dog neural lobe could be released in response to an acute stimulus and that, 10 days after an intraventricular infusion of [^{35}S]cysteine, the hormone in this 'readily releasable' pool was more radioactive than glandular hormone as a whole. In our model the 'readily releasable' pool may be represented by pool 1 (Scheme 1), which is labelled first and contains about 2% of the gland's total hormone. If the primary effect of a stimulus to the gland were to

Table 1. *Analysis of regression table*

Improvement of fit, in terms of total squared error, is shown as the complexity of the model (see under 'Methods') for the passage of vasopressin-neurophysin through the neural lobe increases.

Source of error	$10^{-6} \times$ Total squared error	Degrees of freedom	F ratio
Model $E E$	106.9	96	4.6 ($P < 0.05$)
Model $E EE$	102.0	95	
Reduction by including 2nd exponential	4.9	1	
Model $L E$	88.6	96	4.4 ($P < 0.05$)
Model $L EE$	84.7	95	
Reduction by including 2nd exponential	3.9	1	
Within groups of data	71.6	80	1.04 ($P > 0.25$)
Not explained by $L EE$	14.0	15	
Not explained by $E EE$	30.4	15

Fig. 2. *Computer-derived curve of best fit for the passage of radioactive vasopressin through the neurohypophysis of the rat according to the model described in Scheme 1*

The experimental points from Jones & Pickering (1972) represent the means $\pm$ S.E.M. of four or five observations.

increase k_1 , pool 1 would be rapidly depleted, i.e. it would serve as the 'readily releasable' pool postulated by Sachs. The only problem with this idea is that, in our model, the half-life for the transport of protein from pool 1 to pool 2 ($0.693/k_2$) is 9 h so that, 10 days after radioisotope administration, the hormone in the 'readily releasable' pool would tend to be less radioactive than the total hormone as a whole, whereas Sachs & Haller (1968) found that the hormone released by stimulation *in vitro* or *in vivo* of glands from dogs that had been labelled 9–19 days previously had a higher specific radioactivity than that in the gland as a whole. This discrepancy could be explained in terms of

species differences between the rat and the dog, or it may reflect the different labelling procedures in the two groups of experiments: in the rat, we approached pulse labelling with a single injection of radioisotope, whereas Sachs and his colleagues infused [35 S]cysteine into the third ventricle of the dog for 6 h so that radioactive hormone and neurophysin would have continued to be synthesized for a much longer period. Another possibility is that the size of the 'readily releasable' pool might be smaller under resting conditions than it is when the gland receives strong pharmacological stimuli such as those used by Sachs and his colleagues. In the experiments of Sachs & Haller (1968) mentioned above, although the hormone released from the glands in response to the stimulus had a higher specific radioactivity than the total glandular hormone, that released in the resting period before stimulation had a lower specific radioactivity. This might suggest that a stimulus leads to recruitment of hormone that had most recently entered the 'storage' pool back into the 'readily releasable' pool.

To carry out experiments on the scale necessary for the present investigation, the animals had to be anaesthetized for intracisternal injection. This may cause some stimulation of the hypothalamo-neurohypophysial system but, as the anaesthesia was of very short duration, and as the results of Burford & Pickering (1973) were similar to those of Norström & Sjöstrand (1971) who had injected conscious animals through implanted cannulae, we think that the anaesthesia can have had very little effect.

What does the model mean in terms of the functioning of the gland? Hormone enters the gland in pool 1 (Scheme 1) and may either be released along the route labelled k_1 or be transferred into the 'storage' pool (pool 2) by the process labelled k_2 . For simplicity, we have shown (Scheme 1) the

disappearance from pool 2 as simple release with a rate constant k_3 . However, it is much more likely that release from this pool would occur by passage back into pool 1 and that lysosomal degradation of excess of hormone would also play a part in the disappearance of radioactivity from the storage pool. Stimulation of the gland to release hormone might then be achieved by increasing k_1 [i.e. by accelerating the rate of release from the 'release pool' (pool 1)] and this would lead to the movement back from the storage pool becoming rate-limiting. Thus the phenomenon of the 'readily releasable' pool would be explained by the rapid depletion from the 'releasable pool' followed by a much slower refilling from the 'storage pool'. In considering the possible cellular location of the 'readily releasable' pool, Sachs & Haller (1968) suggested that, amongst others, one possibility was that it may arise from the 'spatial orientation (of hormone) within the axon (e.g. only those neurosecretory granules in close apposition to the neuronal membranes in the nerve terminals can discharge their contents into the perivascular space)'. Our model would fit very well with this hypothesis and, moreover, preliminary studies of electron-microscope radioautographs of rat neural lobes prepared at various times after the intracisternal injection of [^{35}S]cysteine suggest that granules that are newly arrived in the axon termini

are closer to the limiting membrane than those that have been in the gland for some time (Heap *et al.*, 1973).

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References

- Burford, G. D., Jones, C. W. & Pickering, B. T. (1971) *Biochem. J.* **124**, 809–813
- Burford, G. D. & Pickering, B. T. (1973) *Biochem. J.* **136**, 1047–1052
- Heap, P. F., Jones, C. W., Morris, J. F. & Pickering, B. T. (1973) *J. Endocrinol.* **57**, xvi–xvii
- Jones, C. W. & Pickering, B. T. (1972) *J. Physiol. (London)* **227**, 553–564
- Norström, A. & Sjöstrand, J. (1971) *J. Neurochem.* **18**, 2007–2016
- Sachs, H. (1971) *Mem. Soc. Endocrinol.* **19**, 965–973
- Sachs, H. & Haller, E. W. (1968) *Endocrinology* **83**, 251–262
- Sachs, H., Fawcett, C. P., Takabatake, Y. & Portanova, R. (1969) *Recent Progr. Horm. Res.* **25**, 447–491
- Sachs, H., Share, L., Osinchak, J. & Carpi, A. (1967) *Endocrinology* **81**, 755–770
- Sampson, P. (1969) in *BMD Biomedical Computer Programs, X-Series Supplement* (Dixon, W. J., ed.), pp. 177–186, University of California Press, Berkeley
- Thorn, N. A. (1966) *Acta Endocrinol. (Copenhagen)* **53**, 644–654