Simulations of the roles of multiple cyclic nucleotide phosphodiesterases

C. Hugh REYNOLDS*

Biochemical Research Department, The Wellcome Foundation, Temple Hill, Dartford, Kent DA1 5AH, U.K.

(Received 23 July 1981/Accepted 30 September 1981)

1. Simulations were performed using a model for cellular cyclic AMP metabolism involving a hormone-activated adenylate cyclase and two cyclic nucleotide phosphodiesterases with different Michaelis constants. 2. The response curves of cyclic AMP concentration as a function of hormone concentration were affected by regulating the phosphodiesterases. The maximum velocity of the high-affinity phosphodiesterase (V_1) was important in determining the position of the response curve; when V_1 was less than the maximal activity of adenylate cyclase (V_c), sigmoid response curves were readily produced. The maximum attainable concentration of cyclic AMP was determined primarily by V_1 when $V_c < V_1$, and primarily by the activity of the low-affinity enzyme when $V_c > V_1$ ($V_2 \gg V_c$ in all cases). 3. The glucagon-stimulated adenylate cyclase and insulin-stimulated phosphodiesterase of the rat liver plasma membrane were simulated using experimentally determined values for the enzyme-kinetic parameters, and a considerable potential for regulation of the system by insulin was demonstrated. 4. Other possible functions for the regulation of phosphodiesterases are considered, in particular the value of increasing the speed of response to decreases in hormone concentration.

Cyclic nucleotides are important for transmitting the actions of many hormones inside cells. Therefore regulation of the activity of cyclic nucleotide phosphodiesterases (EC 3.1.4.17) could be important for controlling the responses to hormones, complementing the well-established regulation of hormone-sensitive adenylate cyclase. Phosphodiesterases have been described that can be activated by Ca^{2+} and calmodulin (Cheung, 1970; Kakiuchi *et al.*, 1970), other cyclic nucleotides (Beavo *et al.*, 1971) and hormones, including insulin (Loten & Sneyd, 1970; Loten *et al.*, 1978; Malbon & Fain, 1977; Marchmont & Houslay, 1980a, 1981) and glucagon (Allan & Sneyd, 1975), whereas thyroid hormones suppress phosphodiesterase activity (Correze *et al.*, 1976).

Two or more isoenzymes of cyclic nucleotide phosphodiesterase are found in most tissues (Russell *et al.*, 1973; Wells & Hardman, 1977). In some cases, high- and low- K_m enzymes can be separated, and cytosolic phosphodiesterases may have different kinetic properties from membrane-bound enzymes. Homogenates of different tissues from a wide variety of animals (Arch & Newsholme, 1976) gave non-Michaelian kinetics, which suggested either a

mixture of enzymes of high and low K_m , or pronounced negative co-operativity.

Boeynaems *et al.* (1974) showed that the concentration of cyclic AMP in the thyroid gland was raised in response to thyrotropin by a greater factor than was the activity of adenylate cyclase and the accumulation of cyclic AMP was a sigmoid function of the thyrotropin concentration. Two different phosphodiesterases with very different affinities for cyclic AMP were postulated to account for this result. Theoretical studies on the possible roles of multiple isoenzymes of cyclic nucleotide phosphodiesterase have since been presented (Davies & Williams, 1975; Fell, 1980). Erneux *et al.* (1980) used the Hill equation (Hill, 1913) to describe a negatively co-operative phosphodiesterase system; they predicted signal amplification and positive co-operativity of cyclic AMP accumulation.

In the present paper, simulations are reported based on the additive effects of two phosphodiesterases, each of which obey Michaelis–Menten kinetics but have different values for their kinetic parameters. The method is applied to experimental data for the rat liver plasma membrane.

The model

The cytoplasm of an idealized cell is considered to

* Present address: Protein Biochemistry Department, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

contain a uniform concentration of cyclic AMP, which is in a kinetic steady state between synthesis catalysed by a hormone-activated adenylate cyclase, and degradation catalysed by two phosphodiesterases (of high and low affinity).

The rate of synthesis of cyclic AMP is given by:

$$v = \frac{V_c [A]}{K_a + [A]} + B \quad (1)$$

where A represents the hormone that activates adenylate cyclase, B is the basal velocity in the absence of the hormone A , V_c is the maximal hormone-dependent velocity of adenylate cyclase when saturated with hormone and K_a is the apparent Michaelis constant for activation, i.e. the concentration of A that gives half-maximal activation.

In the steady state, v is set equal to the velocity of degradation of cyclic AMP (escape of cyclic AMP from the cell is assumed to be zero, although its kinetic characteristics might well mimic a high- K_m phosphodiesterase and so could be included in the model):

$$v = \frac{V_1 [\text{cyclic AMP}]}{K_1 + [\text{cyclic AMP}]} + \frac{V_2 [\text{cyclic AMP}]}{K_2 + [\text{cyclic AMP}]} \quad (2)$$

This model is similar to previous ones presented by Davies & Williams (1975) and Arch & Newsholme (1976).

Values are assigned for V_c , K_a , V_1 , K_1 , V_2 and K_2 ; then from eqn. (2) the overall rate of degradation is calculated; this is then put into eqn. (1) to give the concentration of A that is required to produce that

Fig. 1. Simulations of steady-state concentrations of cyclic AMP produced by hormone-activated adenylate cyclase and two phosphodiesterases: effect of varying V_1

Values for V_1 were varied, and are shown against each curve. Other parameters were: (a) $V_c = 1$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $K_2 = 100 \mu\text{M}$, $V_2 = 100$; (b) $V_c = 5$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $K_2 = 100 \mu\text{M}$, $V_2 = 100$; (c) $V_c = 1$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $K_2 = 1000 \mu\text{M}$, $V_2 = 100$; (d) $V_c = 5$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $K_2 = 1000 \mu\text{M}$, $V_2 = 100$.

rate of synthesis. Alternatively, when the activity of adenylate cyclase was known, e.g. for basal activity or when hormone was saturating, the concentration of cyclic AMP was calculated from the quadratic equation given by Newsholme (1980).

Results from simulations

Values of parameters

Throughout, K_a was arbitrarily fixed at 1; all hormone (activator) concentrations are given as multiples of K_a and are in effect purely relative. Velocities are given in arbitrary units.

At first, V_1 was fixed at 1, V_2 at 100, K_1 at $0.25 \mu\text{M}$ and K_2 at $100 \mu\text{M}$. The maximum velocity of adenylate cyclase, V_c , was 1 or 5, and basal activity, B , was zero unless otherwise stated. When $V_c = 1$, the low- K_m phosphodiesterase is able to handle most of the cyclic AMP produced and does not become saturated. When $V_c = 5$, the potential exists for excess cyclic AMP to be produced, requiring appreciable activity of the high- K_m phosphodiesterase, which may only be given as the low- K_m enzyme approaches saturation.

In Fig. 1(b) ($V_1 = 5$), the presence of two phosphodiesterases is seen to confer mildly sigmoidal behaviour on the hormone response curves; when $V_1 = 0$ (only a high- K_m phosphodiesterase is

present) the curve is quasihyperbolic. In Fig. 1(a) ($V_c = 1$) no sigmoidicity is apparent, but the upper asymptotes of the curves appear to vary more, as V_1 is increased (see also Table 1).

The sigmoidicity (apparent co-operativity) of the hormone response curve is high if the contribution of the low- K_m phosphodiesterase to the total activity is high, as this enzyme approaches saturation. In Figs. 1(c) and 1(d) the activity of the high- K_m enzyme has been effectively reduced by increasing its K_m 10-fold to 1 mM ; the sigmoidicity and shift of the response curves are now much more marked.

Effects on cyclic AMP concentrations of regulating phosphodiesterases

Varying the value of V_1 , the maximum velocity of the low- K_m enzyme, has a pronounced effect (Fig. 1). For example, in all four cases, doubling V_1 from 1 to 2 at least doubles the concentration of hormone required to produce a given concentration of cyclic AMP. The maximum attainable concentration of cyclic AMP is lowered (Table 1), especially when V_1 is similar to V_c (maximum velocity of adenylate cyclase); this could cause a hormone to be unable, even at high concentrations, to elevate the concentration of cyclic AMP sufficiently to cause a physiological response.

Table 1. *Steady-state concentrations of cyclic AMP at maximal stimulation of adenylate cyclase*

These represent the asymptotic concentrations of cyclic AMP at infinite hormone concentration. Unless otherwise indicated, values of parameters were: $V_1 = 1$; $K_1 = 0.25 \mu\text{M}$; $V_2 = 100$; $K_2 = 100 \mu\text{M}$ or $1000 \mu\text{M}$ (see the Table); $K_a = 1$, and $V_c = 1$ or 5 (see the Table).

	Cyclic AMP concn. in steady-state (μM)			
	$K_2 = 100 \mu\text{M}$		$K_2 = 1000 \mu\text{M}$	
	$V_c = 1$	$V_c = 5$	$V_c = 1$	$V_c = 5$
Varying V_1				
$V_1 = 0$	1.010	5.263	10.101	52.63
0.5	0.644	4.740	5.255	47.15
1	0.391	4.227	1.462	41.73
2	0.175	3.245	0.238	31.10
Varying K_1				
$K_1 = 0.125 \mu\text{M}$	0.297	4.198	1.058	41.70
0.25 μM	0.391	4.227	1.462	41.73
0.5 μM	0.502	4.283	2.002	41.80
Varying V_2				
$V_2 = 50$	0.595	8.761	2.117	87.02
100	0.391	4.227	1.462	41.73
200	0.250	2.096	1.001	20.47
Varying K_2				
$K_2 = 50 \mu\text{M}$	0.251	2.140	—	—
100 μM	0.391	4.227	—	—
200 μM	0.594	8.396	—	—
500 μM	—	—	1.001	20.90
1000 μM	—	—	1.462	41.73
2000 μM	—	—	2.116	83.40

In contrast, altering the value of K_1 only has an appreciable effect at low concentrations of cyclic AMP, i.e. when the low- K_m phosphodiesterase is not saturated. When $V_c = V_1 = 1$ (Figs. 2a and 2c; see also Table 1), the positions and the asymptotes of the curves are affected. In contrast, when $V_c = 5$ (Figs. 2b and 2d) varying K_1 has little effect, and only serves to alter the sigmoidal behaviour at the lower end of the response curve. The effect at saturating hormone concentration is negligible (Table 1).

The high- K_m phosphodiesterase will usually operate at substrate concentrations well below its K_m (K_2), and so the effects of altering its activity through changes in K_2 or V_2 will be very similar. This is shown for asymptotes in Table 1 and is even more valid at lower concentrations of cyclic AMP. Effects on concentration-response curves are illustrated in Fig. 3; they are small when the cyclic AMP concentration is low and hence the high- K_m enzyme is contributing least to cyclic AMP hydrolysis, but they can affect the positions of the curves,

particularly the upper asymptotes. When the cyclase activity is more than twice V_1 , then the high- K_m phosphodiesterase must be performing most of the hydrolysis, and so its regulation will be the more crucial.

Effects of phosphodiesterase inhibitors

Phosphodiesterase inhibitors elevate the steady-state concentration of cyclic AMP at a given activity of adenylate cyclase, which usually leads to an increase in hormonal effects. If an inhibitor is specific for one isoenzyme of phosphodiesterase, and is competitive or non-competitive, then the simulations already presented (Figs. 1–3 and Table 1) also serve to demonstrate the effects of inhibitors.

In practice, inhibitors are not usually specific for one isoenzyme (Amer & Kreighbaum, 1975), and the apparent affinities for competitive inhibitors differ less widely than for the substrate, cyclic AMP. In the special case where a competitive inhibitor has the same value of K_1 for each isoenzyme, a given concentration of the inhibitor will displace the

Fig. 2. Simulations of steady-state concentrations of cyclic AMP produced by hormone-activated adenylate cyclase and two phosphodiesterases: effect of varying K_1

Values for K_1 were varied, and are shown against each curve. Other parameters were: (a) $V_c = 1$, basal cyclase = 0, $K_2 = 100 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$; (b) $V_c = 5$, basal cyclase = 0, $K_2 = 100 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$; (c) $V_c = 1$, basal cyclase = 0, $K_2 = 1000 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$; (d) $V_c = 5$, basal cyclase = 0, $K_2 = 1000 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$.

response-curves upwards by a constant factor, and so will not alter their shape but will enable a given concentration of cyclic AMP to be achieved at a lower concentration of hormone. Table 2 shows this for asymptotes.

Fig. 3. Simulations of steady-state concentrations of cyclic AMP produced by hormone-activated adenylate cyclase and two phosphodiesterases: effect of varying K_2 . Values for K_2 were varied, and are shown against each curve. Other parameters were: (a) $V_c = 1$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$; (b) $V_c = 5$, basal cyclase = 0, $K_1 = 0.25 \mu\text{M}$, $V_1 = 1$, $V_2 = 100$.

Effects of basal activity of adenylate cyclase

In the above simulations, adenylate cyclase has been assumed to be completely inactive in the absence of hormone activator. There is a basal activity of adenylate cyclase in the absence of added hormone, which in assays *in vitro* is usually 20–40% of the maximally stimulated activity (Arch & Newsholme, 1976); the simulations in Fig. 4 show the effects of having 20% of the cyclase activity as basal, with the remaining 80% being hormone-activatable as before. Increasing V_1 can shift the curve back, in part restoring the response; this is more effective when V_c is larger, or K_2 is larger (not illustrated).

Application to liver plasma membranes

Rat liver plasma membranes contain two cyclic AMP phosphodiesterases: an integral high- K_m enzyme and a negatively co-operative peripheral enzyme (Marchmont & Houslay, 1980b; Marchmont *et al.*, 1981). The latter enzyme can be activated by phosphorylation in a cyclic AMP- and insulin-dependent manner (Marchmont & Houslay, 1980a, 1981). The expected activity of phosphodiesterase in the rat plasma membrane was calcu-

Fig. 4. Simulations of steady-state concentrations of cyclic AMP produced by hormone-activated adenylate cyclase and two phosphodiesterases: effect of basal cyclase activity. Values for basal activity (B) and for V_1 are shown against each curve. Other parameters were: $V_c = 1$; $K_1 = 0.25 \mu\text{M}$; $K_2 = 100 \mu\text{M}$; $V_2 = 100$.

Table 2. Effect of a competitive inhibitor on steady-state concentrations of cyclic AMP at maximal stimulation of adenylate cyclase

The inhibition constant, K_i , was the same for each isoenzyme of phosphodiesterase. Values of kinetic parameters of phosphodiesterases were: $V_1 = 1$; $K_1 = 0.25 \mu\text{M}$; $V_2 = 100$; $K_2 = 100 \mu\text{M}$.

[I]	Apparent K_m (μM)		Steady state cyclic AMP concn. (μM)	
	K_1	K_2	$V_c = 1$	$V_c = 5$
0	0.25	100	0.391	4.227
K_i	0.5	200	0.783	8.455
$2 \times K_i$	0.75	300	1.174	12.682

lated from the purification data (Marchmont *et al.*, 1981). Activity for adenylate cyclase, in the presence of 100 μM -GTP with and without glucagon, at the same temperature (30°C) was taken from the work

Fig. 5. Simulations of steady-state concentrations of cyclic AMP at the rat liver plasma membrane in response to glucagon

[A] represents $[\text{glucagon}]/K_g$, where K_g is the activation constant for glucagon. The basal activity of adenylate cyclase was 56 pmol/min per mg of membrane protein and the glucagon-stimulatable activity was 1357 pmol/min per mg. Both curves were obtained from the sum of activities of the peripheral negatively co-operative phosphodiesterase (curve 1, non-phosphorylated; curve 2, phosphorylated) and the intrinsic phosphodiesterase ($K = 58 \mu\text{M}$ and $V = 986 \text{ pmol/min per mg of protein}$). The negatively co-operative phosphodiesterase was described by:

$$\left(\frac{1}{[S]} + P \right) \left(-\frac{1}{v} + M \frac{1}{[S]} + C \right) = Q$$

where

$$P = \frac{V_{m2} - V_{m1}}{V_{m1}K_{m2} - V_{m2}K_{m1}}$$

$$Q = \left(\frac{1}{V_{m1}} - \frac{1}{V_{m2}} \right) P$$

$$M = \frac{K_{m1}}{V_{m1}}$$

$$C = \frac{1}{V_{m1}}$$

V_{m1} and K_{m1} are the apparent maximum velocity and apparent Michaelis constant, obtained from a Lineweaver-Burk plot by extrapolation at very low substrate concentrations; V_{m2} and K_{m2} are obtained similarly from the tangent at very high substrate concentrations. Values were taken from the work of Marchmont *et al.* (1981) and Marchmont & Houslay (1981).

of Houslay *et al.* (1980) and activation was assumed to follow Michaelian kinetics. This system was simulated for both non-phosphorylated and phosphorylated peripheral phosphodiesterase, and is shown in Fig. 5. It includes the intrinsic high- K_m phosphodiesterase (data from Marchmont & Houslay, 1980b) as well as the negatively co-operative phosphodiesterase; the latter was simulated by a hyperbola constrained to give the slopes and intercepts derived by extrapolation from a Lineweaver-Burk plot, but cytoplasmic phosphodiesterases were not included. Only the lower parts of the activation curves are shown in Fig. 5; the upper asymptotes were calculated to be 124 μM and 78 μM with non-phosphorylated and phosphorylated peripheral phosphodiesterase respectively. The calculated basal levels, in the absence of glucagon, were 0.41 μM and 0.19 μM respectively.

Discussion

General considerations of the model

Simulated hormone response curves show the following variations. (a) Variations of maximum cyclic AMP concentrations obtained at saturating hormone concentrations (Tables 1 and 2); this will determine whether a hormone will be able to produce much (or any) physiological response. (b) A variation in hormone concentration required for a given response, i.e. half-maximal cyclic AMP concentration, or a given threshold concentration of cyclic AMP required to produce a response. (c) Variation of shape. The curves are either quasi-hyperbolic or sigmoid; this will determine how the cyclic AMP concentration will change with small changes in hormone concentration. Sigmoid curves will give a greater sensitivity or amplification (Erneux *et al.*, 1980).

Potential for regulation of cyclic AMP response via phosphodiesterases

In simulations, V_1 (the maximum velocity of the high-affinity phosphodiesterase) has been shown to be important. When it is greater than the maximal activity of adenylate cyclase, it will be the primary determinant of the response curve. When V_1 is smaller than the maximal activity of adenylate cyclase, the response curve can be sigmoidal and its location is sensitive to changes in V_1 ; therefore regulation of V_1 will determine the concentration of hormone required to produce an effect (V_2 is always $\gg V_c$). On the other hand, regulation of the low-affinity phosphodiesterase will affect the upper asymptote (maximal concentration of cyclic AMP at saturating hormone concentrations).

Inhibitors of phosphodiesterases increase the steady-state concentration of cyclic AMP at a given activity of adenylate cyclase. For an inhibitor that is

equally effective on both high- K_m and low- K_m phosphodiesterases, the shape of the cyclic AMP-accumulation curves, and hence the hormone concentration for 50% activation, is not changed. Inhibitors with specificity for one of the phosphodiesterases can also alter the shape of the curve.

If there is some adenylate cyclase activity in the absence of hormone activator, the cyclic AMP response to hormone is enhanced at low concentration of hormone. If this basal activity was caused by another hormone that activates adenylate cyclase via a different receptor, then the two hormones could be synergistic, each increasing the response given by low concentrations of the other. Increasing the activity of the low- K_m phosphodiesterase (V_1) could counteract the effect of basal cyclase activity, and this was much more successful when the maximal cyclase activity was in excess of V_1 .

Relevance in vivo

Regulation of phosphodiesterases by various hormones and metabolites has been reported (see the Introduction). An attempt was made to simulate the cyclic AMP response to glucagon of rat liver plasma membranes, and to examine the effect of insulin on this (Fig. 5), by using the data of Houslay and his co-workers (Marchmont & Houslay, 1980b, 1981; Marchmont *et al.*, 1981; Houslay *et al.*, 1980). This might approximate to the situation in rat liver cytoplasm adjacent to the plasma membrane, although the concentration of cyclic AMP would be lower in other parts of the cell due to diffusion and other phosphodiesterases (Fell, 1980). Assayed at $1\mu\text{M}$ -cyclic AMP, phosphodiesterase activity was estimated to be 19% in the plasma membrane, 34% in other membranes and 47% in the supernatant (Westwood *et al.*, 1979), with the supernatant being richer in high- K_m phosphodiesterases; therefore a considerable proportion of the cell's activity, particularly at low concentrations of cyclic AMP, will be located in the plasma membrane. Reported physiological concentrations of cyclic AMP are $0.3\text{--}0.5\mu\text{M}$ in the absence of glucagon (see Marchmont & Houslay, 1980a), rising under maximal glucagon stimulation to $2\text{--}4\mu\text{M}$ (Marchmont & Houslay, 1980a) and up to $16\mu\text{M}$ (Loten *et al.*, 1978). The basal concentrations calculated according to the model presented here are $0.41\mu\text{M}$ with non-phosphorylated peripheral enzyme, or $0.19\mu\text{M}$ for phosphorylated enzyme (however, it is unlikely that the enzyme would be phosphorylated under basal conditions). Under maximal stimulation, calculated concentrations were $124\mu\text{M}$ with non-phosphorylated peripheral phosphodiesterase and $78\mu\text{M}$ with phosphorylated enzyme. Therefore, under basal conditions the agreement is good, but the glucagon-stimulated concentrations are considerably higher;

indeed, the activity of glucagon-stimulated adenylate cyclase reaches 70% of the combined maximum velocities of the membrane-bound phosphodiesterases. Therefore, under glucagon stimulation, a considerable proportion of the cyclic AMP is likely to diffuse away from the plasma membrane and be hydrolysed by phosphodiesterases in the cytoplasm or located on other membranes. Insulin-dependent phosphorylation and activation of the peripheral phosphodiesterase could have a significant effect in counteracting the effects of low concentrations of glucagon (Fig. 5); for example, the concentration of glucagon required to produce $1\mu\text{M}$ -cyclic AMP is increased by a factor of 2.6. Therefore the insulin-dependent activation of liver phosphodiesterase might account quantitatively for the antagonism by insulin of cyclic AMP-mediated stimuli that was proposed by Marchmont & Houslay (1980a).

The stimulation of the negatively co-operative phosphodiesterase resulting from insulin-triggered phosphorylation is most pronounced at low substrate concentrations, and so is analogous to increasing V_1 . Loten *et al.* (1978) have presented a different method for simulating effects of insulin on the glucagon system, based on experimental values for the cyclic AMP concentration, rather than for adenylate cyclase. As the concentration of cyclic AMP decreased, the extra decrease that could be given by insulin-activation of the low- K_m phosphodiesterase became relatively more significant. The maximum velocity of a high-affinity phosphodiesterase has also been reported to be controlled by glucagon (Allan & Sneyd, 1975) and thyroid hormones (Correze *et al.*, 1976). This accords with the prominent role for V_1 found in the simulations. On the other hand, the phosphodiesterases activated by Ca^{2+} and calmodulin (Cheung, 1970; Wells & Hardman, 1977) or by cyclic GMP (Beavo *et al.*, 1971) are usually high- K_m (or relatively high- K_m) enzymes, and these agents have been reported to decrease K_m or increase V_{max} . Activation would have relatively little effect at low concentrations of hormone but could considerably depress the maximal cyclic AMP concentration at saturating hormone, as in Fig. 3.

Other considerations for regulation of phosphodiesterases

Consideration of the kinetics of cyclic AMP metabolism should not be confined to a uniform steady-state model. Fell (1980) has drawn attention to the likelihood of a concentration gradient of cyclic AMP in cells (see above). The turnover-time for cyclic AMP might also be important, and hence the speed of adjustment to a new steady-state concentration of cyclic AMP. The speed of adjustment will be determined by the rate of removal of cyclic AMP, and so will be faster if phosphodiesterase is

activated. Activation of phosphodiesterase would help decrease the concentration of cyclic AMP until it was near the minimum necessary to activate protein kinase (desensitization of adenylate cyclase would also participate in this; Swillens *et al.*, 1980); and this would also hasten the response to hormone withdrawal.

I thank Dr. Miles Houslay, of the University of Manchester Institute of Science and Technology, for interesting discussions and for sharing data before its publication; and Dr. D. A. B. Young and Mr. G. C. Sheppey for help with the algebra describing the liver system.

References

- Allan, E. H. & Sneyd, J. G. T. (1975) *Biochem. Biophys. Res. Commun.* **62**, 594–601
- Amer, M. S. & Kreighbaum, W. E. (1975) *J. Pharmacol. Sci.* **64**, 1–37
- Arch, J. R. S. & Newsholme, E. A. (1976) *Biochem. J.* **158**, 603–622
- Beavo, J. A., Hardman, J. G. & Sutherland, E. W. (1971) *J. Biol. Chem.* **246**, 3841–3846
- Boeynaems, J. M., Van Sande, J., Pochet, R. & Dumont, J. E. (1974) *Mol. Cell. Endocrinol.* **1**, 139–155
- Cheung, W. Y. (1970) *Biochem. Biophys. Res. Commun.* **38**, 533–538
- Correze, C., Auclair, R. & Nunez, J. (1976) *Mol. Cell. Endocrinol.* **5**, 67–79
- Davies, J. I. & Williams, P. A. (1975) *J. Theor. Biol.* **53**, 1–30
- Erneux, C., Boeynaems, J.-M. & Dumont, J. E. (1980) *Biochem. J.* **192**, 241–246
- Fell, D. A. (1980) *J. Theor. Biol.* **84**, 361–385
- Hill, A. V. (1913) *Biochem. J.* **7**, 471–480
- Houslay, M. D., Dipple, I. & Elliott, K. R. F. (1980) *Biochem. J.* **186**, 649–658
- Kakiuchi, S., Yamazaki, R. & Nakajima, H. (1970) *Proc. Jpn. Acad.* **46**, 587–592
- Loten, E. G. & Sneyd, J. G. T. (1970) *Biochem. J.* **120**, 187–193
- Loten, E. G., Assimacopoulos-Jeannet, F. D., Exton, J. H. & Park, C. R. (1978) *J. Biol. Chem.* **253**, 746–757
- Malbon, C. C. & Fain, J. N. (1977) *J. Cyclic Nucleotide Res.* **3**, 315–323
- Marchmont, R. J. & Houslay, M. D. (1980a) *Nature (London)* **286**, 904–906
- Marchmont, R. J. & Houslay, M. D. (1980b) *Biochem. J.* **187**, 381–392
- Marchmont, R. J. & Houslay, M. D. (1981) *Biochem. J.* **195**, 653–660
- Marchmont, R. J., Ayad, S. R. & Houslay, M. D. (1981) *Biochem. J.* **195**, 645–652
- Newsholme, E. A. (1980) *FEBS Lett.* **117**, Suppl., K121–K134
- Russell, T. R., Terasaki, W. L. & Appleman, M. M. (1973) *J. Biol. Chem.* **248**, 1334–1340
- Swillens, S., Lefort, E., Barber, R., Butcher, R. W. & Dumont, J. E. (1980) *Biochem. J.* **188**, 169–174
- Wells, J. N. & Hardman, J. G. (1977) *Adv. Cyclic Nucleotide Res.* **8**, 119–143
- Westwood, S. A., Luzio, J. P., Flockhart, D. A. & Siddle, K. (1979) *Biochim. Biophys. Acta* **583**, 454–466