sentence. When $Q_{\max} \approx 1$, $k' \approx (k_{\text{mod.}} + k'_{\text{mod.}})[E]_0$. The inequality proposed by Varón *et al.* (1992) (their eqn. 5) is therefore entirely inappropriate in precisely those circumstances that they rightly acknowledge to be most relevant.

First-order decay with respect to inhibitor concentration will **strictly** apply only when $Q < Q_{\max} \ll 1$ (Topham, 1987, 1988). However, the requirement for first-order kinetics can still be met experimentally when $Q \approx 1$ by ensuring that $[X]/[E]_0 \gg 1$ during the observation period. This has been demonstrated by computer simulation where it was found that deviations from first-order kinetics are trivial when $Q \approx 1$ provided that $[X]/[E]_0 \gg 1$ (Topham, 1987). There are of course cases where k' is not too large as to preclude experimentation and for which $Q < 1$ (or $Q \ll 1$). In these circumstances it could be argued that the restriction $[X]/[E]_0 \gg 1$ should be relaxed and replaced by either the inequality suggested by Varón *et al.* (1992) or, more consistently, by $k' \gg A[E]_0$. However, both the latter inequalities depend on the unknowns $k_{\text{mod.}}$ and $k'_{\text{mod.}}$. Since the flux ratio will not be known in advance, it is much safer from an experimental viewpoint to retain the requirement that $[X]/[E]_0 \gg 1$ (which only depends on an independent knowledge of k' and $[E]_0$) even when $Q < 1$ (or $Q \ll 1$).

The second example cited by Varón *et al.* (1992) involves what is essentially a stable inhibitor ($t_{1/2} = 19.25$ h). Non-enzymic decay over the time-course indicated on the abscissa of Fig. 1(b) of Varón *et al.* (1992) ($\sim 6.0 \times 10^{-4}$ %) is insignificant by comparison with acceptable limits of experimental error (1–5 %). Using the parameters given by Varón *et al.* (1992) in the legend to Fig. 1(b), $(A[X]_0/k')$ can be calculated to be 50.67 with $k' = 1.0 \times 10^{-5} \text{ s}^{-1}$. However, under these conditions the power series in eqn. (1):

$$\left(\sum_{i=1}^{t-\infty} \left(\frac{A[X]_0}{k'} \right)^i \frac{[1 - (e^{-k't})^i]}{i \cdot i!} \right)$$

does not even begin to converge by the 20th iteration when evaluated using fixed values of $t \leq 0.6$ s. For example, when $t = 0.1$ s, individual terms in the series increase from 5.07×10^{-5} at $i = 1$ through 3.07×10^4 at $i = 10$ to 5.11×10^9 at $i = 20$, an overall change of some 10^{14} -fold. The claim by Varón *et al.* (1992) that the difference between successive values (presumably of $[P]$ although this was not explicitly stated) had disappeared after less than 20 steps in all cases is meaningless because the power series has not simultaneously converged. As in the case of the well-known Maclaurin series expansion of e^x , the power series in eqn. (1) holds for all values of $(A[X]_0/k')$. Indeed, its convergence properties are much better. Thus the reason for the discrepancy between the two curves shown in Fig. 1(b) is simply that Varón *et al.* (1992) have used too low a cutoff for i for their extreme case. In curtailing the calculation at $i = 20$ (or less) the dominant term in eqn. (1) is v^*t . The truncated solution at $i = 20$ gives $[P] \sim v^*t = 8.33 \times 10^{-9} \text{ M}$ when $t = 0.1$ s. Readers wishing to check this value on the lower curve of Fig. 1(b) of Varón *et al.* (1992) should note that the scale depicted on the left-hand ordinate axis is in error by a factor of 10 (given $[E]_0 = 1.0 \times 10^{-8} \text{ M}$ according to the legend).

The reliability of eqn. (1) can be readily ascertained and the numerical simulation result reproduced with little loss in accuracy by setting $k' = 1.0 \times 10^{-4} \text{ s}^{-1}$ [$(A[X]_0/k') = 5.07$, non-enzymic inhibitor decay ~ 0.006 % at 0.6s]. Convergence to three significant figures is now reached in the range $i = 11$ –15. For example, at $t = 0.1$ s, $[P] = 6.67 \times 10^{-8} \text{ M}$, in agreement with the simulation data. In fact the inhibitor is sufficiently stable over the observed time-course that the same result is found using the integrated-rate equation for a stable modifier. This may be derived from first principles or obtained by addition of eqns. (11)

and (18) of Tsou *et al.* (1985) (after setting $B = 0$ for a direct-encounter mechanism). To facilitate comparison with eqn. (1) the equation is given below in terms of the same symbols:

$$[P] = v^*t + \frac{(v - v^*)}{A[X]} \cdot (1 - e^{-A[X]t}) \quad (5)$$

It may be concluded that integrated-rate equations describing time-courses of enzyme modification by unstable modifiers (Gomi & Fujioka, 1983; Topham, 1984, 1985, 1988, 1990; Topham & Dalziel, 1986) are valid provided that experiments are conducted in such a way as to ensure that $[X] \gg [E]_0$ during the observed reaction period. Given this condition, which may be established independently, first-order modifier decay is **effectively** ensured irrespective of the prevailing flux ratio. Rigorous adherence to the argument that first-order decay is only possible when $Q \ll 1$ (Rakitzis, 1987; Varón *et al.*, 1992) overlooks two important practical points. First, there is a need to accommodate cases likely to be encountered experimentally, namely when $Q \approx 1$. Secondly, the flux ratio depends on unknown kinetic parameters describing the modification reaction and is therefore itself also unknown in real situations.

The apparent discrepancies illustrated in Fig. 1(b) of Varón *et al.* (1992) can be explained by the failure of their computer program to carry out rigorous tests for convergence. Although there are no theoretical limits on the magnitude of $(A[X]_0/k')$, the power series in eqn. (1) will not converge quickly when $(A[X]_0/k') > 5$. When this threshold is exceeded because $k' \rightarrow 0$, it is more sensible to use the analogous equation for a stable modifier.

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Low concentrations of adenine nucleotides enhance the receptor binding of inositol 1,4,5-trisphosphate

The Ca^{2+} -mobilizing second messenger inositol 1,4,5-trisphosphate (InsP_3) acts on specific binding sites, first described in hepatocytes and polymorphonuclear leukocytes [1], pituitary [2], and adrenocortical microsomes [3]. The receptor which was purified [4], cloned and sequenced from rodent cerebellum [5,6] is a homotetramer of M_r -313000 protein subunits, each of which contains two adenine nucleotide binding sites (Gly-Xaa-Gly-Xaa-Xaa-Gly) at amino acid residues 1773–1778 and 2016–2021. In non-neuronal tissues the deletion of a 120-nucleotide segment designated SII (at amino acid residues 1687–1732) [7] gives rise to the appearance of a third nucleotide binding site. The presence of ATP-binding sites has been confirmed by photoaffinity labelling

Table 1. Effect of adenine nucleotides on the binding of [³²P]InsP₃ by pituitary microsomes

Pituitary microsomes [2] were incubated at 0 °C in a cytosol-like medium (10 mM-NaCl, 100 mM-KCl, 0.513 mM-MgCl₂, 1 mM-NaH₂PO₄, 10 mM-Hepes, 1 mM-EGTA, 0.33 mM-CaCl₂, 1 mg of bovine serum albumin/ml, pH 7.0) containing about 150 Bq/500 µl (≈ 1 nM) of [³²P]InsP₃ (DuPont NEN), in the absence or presence of various concentrations of ATP (Sigma), ADP or adenosine 5'-[βγ-imido]triphosphate (AMP-PNP) (Pharmacia). Incubation was terminated after 5 min by filtration (for details see [2]). Binding values, corrected for non-specific binding (40–80 c.p.m./mg of protein) and expressed relative to specific binding observed in the absence of nucleotides (246 ± 30 c.p.m./mg of protein, *n* = 9) are shown. Values are means ± S.E.M. (ATP) or means ± range (ADP and AMP-PNP). Each experiment was done in duplicate. **P* < 0.001, as compared to bound without nucleotide.

Nucleotide added	Concentration (µM)	<i>n</i>	Ratio ($\frac{\text{bound with nucleotide}}{\text{bound without nucleotide}}$)
No addition			1
ATP	1	8	1.315 ± 0.038*
	10	8	1.016 ± 0.049
	100	8	0.408 ± 0.033
ADP	1	2	1.188 ± 0.052
	10	2	1.130 ± 0.060
	100	2	1.079 ± 0.061
	1000	2	0.588 ± 0.282
AMP-PNP	1	2	1.77 ± 0.063
	10	2	1.207 ± 0
	100	2	1.145 ± 0.016

[8]. The InsP₃ receptor functions as a Ca²⁺-release channel [9], modified by ATP in a concentration-dependent manner. ATP competes with InsP₃ for the InsP₃ binding sites [2,10–12]. This competition contributes to the high EC₅₀ (the concentration required for half-maximal effect), measured in Ca²⁺ release studies in the presence of ATP as opposed to the much lower dissociation constant (*K_d*) of the receptor-ligand complex. However, ATP, as usually applied in the range of 10⁻⁶–10⁻⁴ M, facilitates rather than inhibits the action of InsP₃ in several systems. It enhanced InsP₃-induced Ca²⁺ release in smooth muscle cells [13,14] as well as InsP₃-activated Ca²⁺ conductance in liposomes [15] and planar lipid bilayers [8,16]. The mode of this enhancer action has not yet been elucidated.

We performed InsP₃ binding studies on bovine pituitary microsomes (35000 *g* pellet) exactly as described [2]. In these studies 5 nM unlabelled InsP₃ reduced the specific binding of [³²P]InsP₃ (measured without adding unlabelled ligand) to 24.3 ± 8.1 % (S.E.M., *n* = 9). Confirming previous observations [2], ATP at 10⁻⁴ M reduced the binding of [³²P]InsP₃; however, at 1 µM it enhanced the binding to 131.5 ± 3.8 % of the control (*P* < 0.001, *n* = 8) (Table 1).

Low concentrations of ADP also enhanced the binding of [³²P]InsP₃, albeit the effect was smaller than that of ATP (Table 1). In this respect it is worthwhile to recall that ADP is a potent competitor of ATP for [α-³²P]ATP binding to the purified InsP₃ receptor [8]. A non-hydrolysable analogue of ATP increased the opening probability of InsP₃-gated channels of aortic sarcoplasmic reticulum [16]. In accordance with this observation, in the present study the non-hydrolysable analogue adenosine 5'-[βγ-imido]triphosphate (AMP-PNP; Pharmacia) also facilitated the binding of [³²P]InsP₃ (Table 1). Considering that InsP₃ binding was enhanced by non-phosphorylating nucleotides, the enhancement may not be attributed to the recently described autophosphorylation of the receptor [17].

Our observations explain the biphasic effect of ATP on the InsP₃ receptor. When present at physiological concentrations the nucleotide competes with InsP₃ for InsP₃-binding sites and increases the EC₅₀ of InsP₃. At low concentrations ATP acts at specific nucleotide-binding sites and enhances the binding of InsP₃. The elucidation of the physiological significance of these opposing effects requires further studies.

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