

TABLE IV

*Experimental measurements of the rate of activation of catalytic subunits of PDE per Rh\**

Species	<i>T</i> (°C)	[GTP] (μM)	<i>f</i> <sub>φ</sub>	<i>v</i> <sub>RP</sub> (PDE* s <sup>-1</sup> per Rh*)	Reference
1. Frog	21	130	< 10 <sup>-5</sup>	155	[203]
2. Frog, Toad	21	125	< 10 <sup>-5</sup>	140	[120]
3. Toad	24	1000	< 10 <sup>-5</sup>	135	[11]

Each of these investigations employed pH-electrode measurements of proton production by the PDE-catalysed cyclic GMP hydrolysis reaction, cyclic GMP  $\leftrightarrow$  5'GMP + H<sup>+</sup> (p*K*<sub>a</sub> = 6.5), in a suspension of rod disc membranes; the basic methodology is described in Ref. 203. In these experiments flashes isomerizing fractions *f*<sub>φ</sub> of the rhodopsin, such that *f*<sub>φ</sub> < 10<sup>-5</sup> (in frog and toad disc membrane suspensions) produce cyclic GMP hydrolysis curves whose steady-state rates are a linear function of *f*<sub>φ</sub>. Specifically, the time course of the rate of proton production is well described by the general form  $V = V_p[1 - \exp(-t/\tau)]$ , where  $\tau = 2-5$  s when only GTP is present, and *V*<sub>p</sub> is the peak hydrolytic velocity for a flash isomerizing a fraction *f*<sub>φ</sub> of the rhodopsin, and the peak hydrolytic velocity obeys the relation  $V_p = V_{max} [1 - \exp(-f_\phi N_D)]$ , where *f*<sub>φ</sub> is the fraction of rhodopsin isomerized, *N*<sub>D</sub> is the 'domain size' (typically 50000 to 90000 rhodopsins), and at room temperature *V*<sub>max</sub> is typically 8-12 mol cGMP s<sup>-1</sup> per mol total Rh in the reaction cuvette. This value of *V*<sub>max</sub> (per mol total rhodopsin in the reaction volume) may be converted to 2*k*<sub>cat</sub>, the value per holo-PDE by dividing by the ratio of PDE/rhodopsin approx. 1/150 in amphibia (Table I), to give about 2000 s<sup>-1</sup> (see Table V). From the two relations above, the rate *v*<sub>RP</sub> of PDE catalytic subunit activation at early times and for dim flashes is found to be 2(1/150)(*N*<sub>D</sub>/τ) PDE\* subunits s<sup>-1</sup> per Rh\*; this formula was used to derive the values in Table IV.