in the strained literalism of Cornish-Bowden & Koshland (1975) and Bardsley (1977).

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Effect of ATP on receptor binding of inositol 1,4,5-trisphosphate

Inositol 1,4,5-trisphosphate (InsP_3), a second messenger formed in response to calcium-mobilizing hormones, neurotransmitters and growth factors, induces the release of Ca^{2+} from a non-mitochondrial intracellular pool (for review, see Berridge, 1987). Binding of InsP_3 to high-affinity intracellular receptors is specific, reversible, saturable and correlates with the biological response (Spät *et al.*, 1986a). Studies on microsomal preparations, however, showed that the dissociation constant (K_d) of the receptor–ligand complex (at 4 °C) is about two orders of magnitude lower than the concentration required for half-maximal Ca^{2+} release (EC_{50}) (at 30 or 37 °C) (liver: Spät *et al.*, 1986a; Lukács *et al.*, 1987; adrenal cortex: Guillemette *et al.*, 1987; pituitary: Kiesel *et al.*, 1987; Spät *et al.*, 1987). Recently we observed that ATP displaces [^{32}P] InsP_3 from its binding sites in a rat pituitary microsomal preparation (half-maximal displacement was attained at 90 μM) (Spät *et al.*, 1987). This observation has been confirmed by Guillemette *et al.* (1988) and a similar competition has been described also

for an adrenocortical microsomal suspension (Guillemette *et al.*, 1987). Considering that ATP is applied in millimolar concentrations for Ca^{2+} -transport studies, we presumed that its binding to InsP_3 receptors may contribute to the discrepancy between K_d and EC_{50} .

In an attempt to elucidate the mode of action of ATP on InsP_3 binding, in three experiments we compared the effect of two different types of ATP on Ca^{2+} metabolism and InsP_3 binding of bovine pituitary microsomal vesicles (35000 *g* pellet; for methods, see Spät *et al.*, 1987). In these preparations 5 nM unlabelled InsP_3 reduced the specific binding of [^{32}P] InsP_3 to $22.6 \pm 3.8\%$ (S.E.M.) of the maximum. Microsomal Ca^{2+} uptake did not depend on the type of ATP applied (results not shown). On the other hand, there was a striking difference in their effect on receptor binding. ATP produced by Sigma (type A-7894) displaced labelled InsP_3 from the binding site; at 100 μM the binding was $42.3 \pm 5.8\%$ of maximum. (In several previous experiments both this type of ATP and also type A-5394 significantly displaced the labelled ligand; results not shown.) ATP produced by Pharmacia (type 27-1006-0) had no significant effect on the binding; at 100 μM the binding was $112.2 \pm 11.7\%$ of the control.

These results show that ATP itself does not interfere with the binding of InsP_3 to its specific receptor, and the previous observations of such a phenomenon may have been due to an impurity in some preparations. The cause of the discrepancy between K_d and EC_{50} remains to be further examined.

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