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Letter to the Editors

Concerning the Concentration of ATP in the Junctional Cleft that is Threshold for Potentiation of Cholinergic Sensitivity

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Dr. Ribeiro has raised a question which was inadequately discussed in my paper: Are there physiological conditions under which the concentration of endogenously released ATP in the juctional cleft is sufficiently high to produce the effects on postjunctional sensitivity to ACh which I have described for exogenously applied ATP?

Sensitivity to ACh is potentiated by ATP and ADP at concentrations in the millimolar range (2 to 10 mM) on diaphragm muscles which have not been previously enzymatically digested. I postulated that the biochemical mechanism underlying this potentiation occurred in an enclosed volume of extracellular space—the junctional cleft. I attempted to make this space more "available" to bath-applied nucleotides by predigesting the preparations in Ringer-Locke solutions of collagenase (1.0 mg/ml) and protease (0.1 mg/ml). Digestion for 140 min causes a separation of the nerve endings from their postjunctional moorings called "disjunction" (Betz & Sakmann, 1971). Using only half of this digestion time, 70 min, the threshold concentration of ATP to produce potentiation is reduced to 0.2 mM (*see* Fig. 6). This is only twice the cleft concentration which Silinsky calculates for endogenous release after a brief, repetitive stimulation—0.1 mM (Silinsky, 1975). Digestion times longer than 70 min might have further reduced this threshold, but were avoided for the following reason: these preparations fibrillate when exposed to concentrations of ATP above 1.0 mM. Fibrillations disrupt the measurement of sensitivity and a full dose-response curve cannot be obtained. With a reduction of the digestion time to 35 min, fibrillation does not occur with ATP concentrations up to 5 mM allowing measurement of the potentiation phenomenon to saturation. Using the 35 min dose-response curve, I proceeded to investigate the Ca^{++} dependence of the phenomenon in the most sensitive concentration range (at 1.0 mM ATP where potentiation is about half-maximal) and to compare the effects of other

nucleotides at 1.0 mM and at 5.0 mM (saturation). Thus the "pharmacodynamic" properties of the preparation led me to use a relatively high concentration and minimal predigestion.

The above experiments suggest the existence of a pharmacological "barrier" to nucleotide-induced potentiation which is reduced by enzymatic digestion. The enzymatic digestion of a simple diffusion barrier, which is presumably the first stage of disjunction, is a reasonable mechanism for this reduction. De-activation of an extracellular, Ca^{++}-activated nucleotidase by proteolytic digestion would also reduce the barrier by preventing the degradation of ATP and ADP to AMP which does not potentiate sensitivity. (The two-fold enhancement of potentiation by 1.0 mM ATP in Ca^{++}-free solution shown in Fig. 9*B* supports this hypothesis.) By either mechanism the threshold concentration of ATP in the cleft itself would be well below the threshold concentration in the bath. I conclude that the prejunctional and postjunctional membranes are *both* responsive to physiological concentrations of extracellular ATP, albeit with opposite effects on neuromuscular transmission. Furthermore, the prejunctional (Silinsky & Hubbard, 1973) and postjunctional (Meunier, Israël, & Lesbats, 1975) membranes are both independently capable of releasing ATP when stimulated. The "trophic" effect mediated by ATP in this system is obviously a complex intercommunication between these membranes which, from a teleological point of view, serves to maintain an optimal functional state for neuromuscular transmission over a wide range of physiological conditions.

References

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