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Effect of antibiotics and selective inhibitors of ATP on intestinal slow waves

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JOB, DONALD D. *Effect of antibiotics and selective inhibitors of ATP on intestinal slow waves.* Am. J. Physiol. 220(2): 299–306. 1971.—Antibiotics selective for mitochondrial ion fluxes and selective inhibitors of mitochondrial synthesis of ATP were studied in order to elucidate the relationship between ATP production and the amplitude and frequency of intestinal slow waves. The slow-wave amplitude was initially enhanced then inhibited by valinomycin and monensin at low concentrations. These agents also increased or decreased frequency depending on the concentration. Uncouplers and inhibitors of electron transport—dinitrophenol, pentachlorophenol, and oligomycin; and cyanide, antimycin A, rotenone, and thenoyl trifluoroacetone—all inhibited slow-wave amplitude. The slow-wave frequency, in general, was not changed by uncouplers, but was by electron-transport inhibitors in some instances. Slow waves could be produced after antimycin A inhibition by adding electron donators. The system described has the characteristics of a Van der Pol relaxation oscillator.

intestinal slow waves; energetics of slow waves; slow-wave frequency; smooth muscle electrophysiology

THE SLOW WAVES of the cat jejunum have been attributed to the cyclical passive influx and active efflux of sodium ions (20, 24). The origin of this cyclical process was suggested by these authors to be related to ATP synthesis by mitochondria. It was of interest, therefore, to test the effect of additional selective inhibitors of ATP synthesis on slow waves.

Considerable attention has been paid in previous investigations to factors that affect the slow-wave amplitude. No investigation has been reported that deals systematically with factors that affect slow-wave frequency. Previous investigators have shown that the frequency of the intestinal slow waves decreases markedly with a decrease in temperature (5, 12, 20). No other factors have been reported to exhibit such a marked influence on slow-wave frequency. It was the additional aim of these studies, therefore, to look specifically for effects of various inhibitors on slow-wave frequency.

The relations between temperature and frequency and the relative stability of the frequency against various inhibitors are similar to the characteristics of the periodic oscillations in many mitochondrial preparations (9, 11). These oscillations in swelling and contractions as measured by light scattering have been shown to be related to fluxes of K^+, Na^+, and H^+ ions across the mitochondrial membrane (9, 27). These oscillations were induced by adding the macrolide actins, valinomycin, or gramicidin to the mitochondrial suspension (16, 27). The oscillations were sensitive to changes in osmolarity, temperature, and pH. The period of oscillations ranges from 30 to 90 sec. The final aim of the present study was to determine whether a relationship existed between oscillations in the mitochondria and oscillations in the membrane potential.

Several antibiotics that have been shown to selectively block ion transport across mitochondria were used to correlate mitochondrial fluxes with the intestinal slow waves. There are two groups of antibiotics. One induces cation uptake and swelling; the other reverses this process. Valinomycin induces swelling of mitochondria consequent to the selective uptake of potassium ions (27). The monactin homologues, monactin and dinactin, and the gramicidins A–D also induce cation-requiring swelling of the mitochondria, but are less selective for potassium (16, 26). In the second group, monensin (14) and nigericin (15) will block or reverse the swelling and cation uptake induced by valinomycin. Monensin and nigericin are reported to block the transport of both Na^+ and K^+.

The selective inhibitors of ATP utilization were chosen either for their ability to block electron transfer at specific sites or their ability to uncouple oxidative phosphorylation. The inhibitors of electron transport were amytal and rotenone, which inhibit electron transfer from nitotinamide adenine dinucleotide (NAD^+) to the coenzyme Q complex (CoQ) between the flavoprotein and cytochrome *b* (8, 11); antimycin A, which blocks electron transfer between CoQ-cytochrome *b* complex and cytochrome *c* (10); thenoyl trifluoroacetone, which blocks electron transfer between succinate and CoQ complex after the flavoprotein (11); and cyanide, which blocks the electron transfer from cytochrome *a* to oxygen (2). The uncouplers studied were dinitrophenol (DNP) and pentachlorophenol, which block the formation of the high-energy intermediate (23, 32); and oligomycin, which presumably blocks the conversion of a high-energy intermediate to ATP (18, 13). Dinitrophenol has previously been reported to depress intestinal slow waves (5, 6). The principal basis for assuming that a given inhibitor was acting where it is reported to act in cell-free systems was that usually several structurally different compounds within a group of inhibitors were studied; and, if all compounds in this group caused a similar response in the intact system, it was inferred that they were acting on the same site. The results from only one representative of each group have been presented. In the case of the antibiotics, it is likely that the plasma membrane is also affected since their primary action is on membranes.