Selective Inhibition of Na,K-ATPase by Progesterone Analog Megestrol-17-Acetate and Structural Requirements to Non-Genomic Interaction Between Progestins and Na/K-ATPase.

Pimentel C.M.; Fontes, C.F.L. 1

1- Lab. de Est. e Reg. de Proteínas e ATPases, Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, Brazil.

Introduction—We studied the non-genomic effects of progesterone on Na/K-ATPase, which behaved as mixed-type inhibitor, without conformational selection (E1 or E2). We aimed to investigate whether megestrol-17-acetate (M17Ac) is a conformation-selective inhibitor and what could be the structural requirements for steroid binding and inhibition, testing M17Ac and other steroids.

Material and methods—Na/K-ATPase determinations of a purified pig kidney medulla preparation were through modified Fiske-Subbarow method. Kinetic data were fitted through Dixon/Cornish-Bowden analysis. Controlled proteolysis with M17Ac was performed to detect Na/K-ATPase E1–E2 states, as described (Tribuzy et al. 2002, A.B.B. 399:89-95). Measurements of pNPPase were as (Dey et al., 2010, Life Sci. 86:473-481).

Results and Discussion - Boldenone (17-OH), progesterone-3-o-carboxymethylxoxime (17-Ac, but with a bulky group at 3-position) and Medroxiprogesterone-17-acetate; were only marginal inhibitors with respectively 31.84, 7.97 and 29.84 % inhibition. However, M17Ac was a potent inhibitor (94% inhibition) behaving as an unusual mixed inhibitor (anomalous Dixon plots) where the substrate increment augments the inhibitor affinity. When the Na/K ratio was shifted from 6.5 (optimal) to 15 favoring E1-state, M17Ac potency was unaltered (92.56% inhibition), with mixed-inhibition profile (Ki=4.74 µM and K'i= 9.58 µM). If the Na/K ratio is decreased (favoring E2-state) no significant inhibition was seen. This was confirmed by pNPPase experiments in which up to 50 µM of M17Ac were ineffective. Proteolysis also suggests a selective E1 inhibition by M17Ac, in which fragments of 78 kDa are stabilized and 58 kDa/39-43 kDa (typical from E2-conformers) were reduced. Conclusion—17-acetate in ring D seems important for the interaction; but planar/tetrahedric 6-methyl of ring B was decisive in between medroxiprogesterone-17-acetate and M17Ac action. Moreover, the Ki for M17Ac has a parallel with levels found in orally-administered patients (1.96 µM). It suggest a possible physiological interaction notably when M17Ac is used as contraceptive or anabolic treatment (AIDS patients).

Supported by CNPq