Farmacologia Cardiovascolare dei Donatori di Ossido d'Azoto (NO): Prospettive Terapeutiche
6° Convegno Monotematico SIF – Milano, 31 Maggio 2002

MODULATION OF VENTRICULAR CALCIUM CURRENT BY NO/O_2^--DONORS

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We investigated the potential involvement of $ONOO^-$ in the modulation of calcium current (I_{Ca}) in isolated guinea pig ventricular myocytes with the whole-cell patch clamp technique and with cyclic AMP (cAMP) measurements. Because of the instability of $ONOO^-$ at physiological pH, we induced an increase in its intracellular levels by using donors of the precursors, nitric oxide (NO) and superoxide anion (O_2^-). When cells were exposed to high concentrations of NO donors (SpermineNONOate, 300µM, or SNAP, 300 µM) basal calcium current significantly increased (respectively 50.3 ± 4.6 %, n=7 and 46.2 ± 5 %, n=13). Applications of high doses of the superoxide anions donor Pyrogallol (300 µM) also stimulated basal I_{Ca} (44.6 ± 2.8 %, n=11). At lower concentration SpermineNONOate(10 nM) and Pyrogallol (1 µM), although separately uneffective on I_{Ca}, enhanced basal calcium current when applied together (33.5 ± 0.7 %, n=7). The simultaneous donor of both O_2^- and $ONOO^-$, SIN-1 (500 µM), also stimulated basal calcium current (23.6 ± 3 %, n=9), with a cGMP independent mechanism. Mn(III)tetrakis(4–Benzoic acid)porphyrin chloride (MnTBAP) 100µM, a $ONOO^-$ scavenger, although increased by itself the basal calcium curent (52 ± 5.8 %, n=10) reversed the stimulatory effect of SIN-1 on I_{Ca} (–0.6 ± 4.1%, n=4). Intracellular cAMP level, measured with an enzime immuno assay (EIA), was unaffected by SIN-1 while was enhanced by blocking the NO-cGMP pathway with the NO synthase inhibitor L-NMMA. These results suggest that peroxynitrite donors increase cardiac calcium current without the involvement of cAMP and cGMP.