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EFFECTS OF W-7 ON CATECHOLAMINE RELEASE AND $^{45}Ca^{2+}$ UPTAKE IN CULTURED ADRENAL CHROMAFFIN CELLS.

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Summary

Effects of N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), a calmodulin antagonist, on catecholamine (CA) release and $^{45}Ca^{2+}$uptake were studied using cultured bovine adrenal chromaffin cells. W-7 inhibited the carbamylcholine (CCh)-evoked CA release and $^{45}Ca^{2+}$ uptake in a concentration-dependent manner. The inhibitory effect of W-7 on CCh-evoked CA release was not overcome either by an increase in extracellular calcium or CCh concentration. Although W-7 inhibited the high K^+-evoked CA release and $^{45}Ca^{2+}$ uptake, potency of the drug was approximately 50-100 fold less than when inhibiting the CCh-evoked CA release and $^{45}Ca^{2+}$ uptake. The inhibitory effects of W-7 were observed both in norepinephrine release and epinephrine release. Moreover, W-7 inhibited the CCh-evoked $^{45}Ca^{2+}$ efflux. These results suggest that the inhibition of CA release by W-7 in adrenal chromaffin cells is mainly due to its inhibition of calcium uptake. W-7 may influence the linkage between acetylcholine-receptor and calcium uptake with higher potency than depolarization-dependent calcium entry.

Adrenal medullary chromaffin cells secrete CA by a calcium dependent exocytotic process(1). There is increasing evidence that calmodulin plays important roles in various calcium-dependent cell functions(2,3). It seems possible that calmodulin plays some roles in secretory function of adrenal chromaffin cells, since calmodulin has been detected in chromaffin cells (4). In addition, several investigators have reported recently that trifluoperazine and some other calmodulin antagonists, inhibit CA release in adrenal chromaffin cells (5-8). The mechanism of its inhibitory action, however, is still controversial on the point of action on calcium flux.

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Hidaka et al. recently reported that W-7, a new calmodulin antagonist chemically unrelated to trifluoperazine, was found to easily enter the intracellular space. They claimed that W-7 is a more selective antagonist of calmodulin than trifluoperazine and chlorpromazine(9,10). Therefore, in this study we examined the effects of W-7 on CA release and calcium uptake evoked either by CCh or high K^+ using cultured adrenal chromaffin cells.

Materials and Methods

Primary culture of adrenal chromaffin cells: Fresh bovine adrenal glands were obtained from a local slaughterhouse, and chromaffin cells were isolated by treatment with 0.04% collagenase as described by Kumakura et al(4). The purified chromaffin cells were cultured by the method described by Kilpatrick et al.(11) with slight modification. The cells were plated on a 16 mm diameter well of a 4-well plastic multidish at a concentration of 3-5 x 10^5 cells per well in Dulbecco's modified Eagle's Medium, supplemented with 10% fetal calf serum, and cultured at 37°C in an atomosphere of 95% air/5% CO_2. The culture medium contained the following antibiotics ; penicillin (100 units/ml), streptomycin (100 µg/ml), gentamicin (40 µg/ml) and mycostatin (25 units/ml). The medium also contained fluorodeoxyuridine (10 µM), cytosinearabinoside (10 µM) and uridine (5 µM) to prevent the proliferation of non-neuronal cells, and was replaced every 3 to 4 days. Cell viability estimated by a trypan blue exclusion test was above 95% after 10 days of culture. The cells were used for experiments between 4 and 10 days of culture.

CA release and CA assay: Cultured cells were first washed with 95% O_2 and 5% CO_2 saturated Krebs-Ringer bicarbonate glucose (KRBG) buffer (pH. 7.2-7.4) of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.2, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11 containing 0.5% bovine serum albumin, and were incubated with one milliliter of the same buffer. To determine the effects of W-7 and verapamil, the cells were preincubated at 37°C for 10 min with different concentrations of each drug. After preincubation, CCh (final concentration: $3x10^{-4}$ M) or KCl (final concentration: 56 mM) was added to the medium and the incubation was continued for another 3 min to measure catecholamine release. When the cells were stimulated with A23187, the incubation was continued for 5 min. Cellular catecholamines (norepinephrine and epinephrine) and released catecholamines (norepinephrine and epinephrine) in the medium were extracted with 0.4 N perchloric acid and analyzed by a high performance liquid chromatography (Waters Assoc., Milford. Massachusetts) equipped with an electrochemical detector (Bioanalytical Systems Inc., W. Lafayette, Indiana) (12). Released catecholamines were expressed as; 1) The percent of the total cellular content, 2) the percent of the maximum release evoked by $3x10^{-4}$ M CCh or 56 mM KCl.

Measurement of $^{45}Ca^{2+}$ uptake: After preincubation of the cells in 0.45 ml KRBG buffer in the presence and absence of W-7 at 37°C for 10 min, 0.05 ml KRBG buffer containing $^{45}CaCl_2$ (1 µCi/well) and each secretagogue was added to each well. After 1 min, the radioactive medium was removed to stop the uptake, and the cells were immediately washed 4 times with ice-cold KRBG buffer. The cells were solubilized in 1% triton X-100 solution in water for 30 min and the radioactivity was determined.