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MONENSIN-INDUCED INFLUX OF ^{22}Na AND THE RELEASE OF CATECHOLAMINES IN CULTURED BOVINE ADRENAL MEDULLA CELLS AND ISOLATED CHROMAFFIN GRANULES

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Abstract—In cultured bovine adrenal medulla cells, monensin caused the release of catecholamines simultaneous with the influx of ^{22}Na to the cells. The release of catecholamines by monensin was dependent on Na but not on Ca in the medium. Release of catecholamines and the influx of ^{22}Na caused by monensin were not inhibited by tetrodotoxin. Monensin did not cause the release of dopamine β-hydroxylase from the cells showing that monensin caused the release of catecholamines by a nonexocytotic mechanism. Similarly, in isolated chromaffin granules, monensin caused Na-dependent release of catecholamines, simultaneously with the influx of ^{22}Na to the granules. Basing on these findings, monensin seems to cause a nonexocytotic release of catecholamines by acting as an Na ionophore both at cell membranes and chromaffin granule.

Monensin is an antibiotic isolated from *Streptomyces cinnamonensis* and one of the typical carboxylic ionophores which shows selectivity to monovalent cations especially to Na. Monensin has been shown to cause the release of catecholamines from cultured bovine adrenal medulla cells [1] and transplantable rat pheochromocytoma PC-12 cells [2]. Release of catecholamines caused by monensin was shown to be dependent on Na but not on Ca in the medium. The physiological secretagogue acetylcholine, and another Na ionophore veratridine, cause the release of catecholamines which depends on Na and Ca in the medium [3–5]. Since Ca plays an important role in the release of catecholamines, the rise in cellular Na concentration by monensin has been considered to mobilize endogenous Ca and this Ca triggers the release of catecholamines [1]. In PC-12 cells, monensin caused the release of catecholamines also from the catecholamine storing chromaffin granules [6].

In this paper, to study the mechanism of catecholamine release by monensin, we have investigated the release of catecholamines from the cultured bovine adrenal medulla cells and isolated chromaffin granules and simultaneously measured ^{22}Na influx into the cells and chromaffin granules. We also compared the effects of monensin with those of veratridine.

MATERIALS AND METHODS

Bovine adrenal medulla cells were isolated and cultured [4, 7]. Influx of ^{22}Na into the cultured cells and release of catecholamines from the cells were examined [3]. Efflux of ^{45}Ca was also examined as reported elsewhere [8]. Chromaffin granules were isolated in isotonic sucrose (270 mM)–50 mM Tris–HCl buffer (pH 7.4) by millipore filtration [9], and were suspended in 225 mM sucrose–25 mM NaCl–50 mM Tris–HCl (pH 7.4), and incubated with $^{22}NaCl$ (1 μCi) in the presence and absence of monensin for 15 min at 37°. After incubation, chromaffin granules were sedimented by centrifugation. Catecholamines released into the medium were absorbed to alumina and assayed by ethylenediamine condensation method [10]. Dopamine β-hydroxylase activity was measured according to Pisano *et al.* [11]. Sedimented chromaffin granules were solubilized with 1% of Triton X-100 and the radioactivity of ^{22}Na was counted by liquid scintillation counter.

Monensin and veratridine were obtained from Sigma. $^{22}NaCl$ (4–6 Ci/mmol) was from Amersham. Collagenase and trypsin inhibitor used in cell isolation were from Sigma. Eagle MEM culture medium was from Nissui. Other chemicals were from Nakarai Chemicals of analytical grade.

RESULTS

Effects of monensin on cultured bovine adrenal medulla cells

As has been already reported [1], monensin caused slow and prolonged release of catecholamines from the cultured bovine adrenal medulla cells. In five hours, more than 80% of cellular catecholamines were released into the medium by 0.1 μM of monensin. The release was not observed in Na-free medium, but occurred in Ca-free medium. Figure 1 shows the concentration–response curve for monensin of the release of catecholamines and the influx of ^{22}Na. Monensin at concentration of 0.1–0.3 μM showed the maximal release of catecholamines and the influx of ^{22}Na. The influx of ^{22}Na caused by

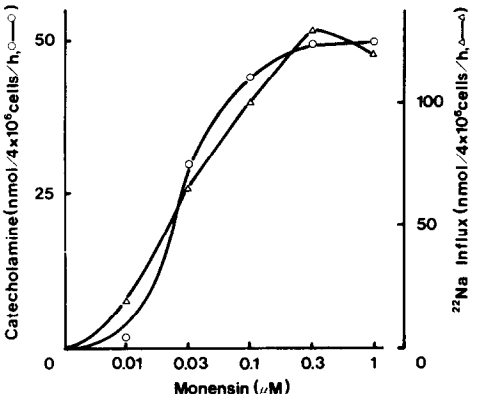

Fig. 1. Dose–response relationships for the monensin-induced influx of ^{22}Na to the cultured bovine adrenal medulla cells and the release of catecholamines. Cultured bovine adrenal medulla cells (4×10^6 cells/dish) were incubated with $^{22}NaCl$ under various concentrations of monensin in Krebs–Ringer phosphate buffer containing 0.5% bovine serum albumin. The buffer had the following composition (mM); NaCl 154, KCl 5.6, $MgSO_4$ 1.1, $CaCl_2$ 2.2, NaH_2PO_4 0.85, Na_2HPO_4 2.15 and glucose 10 (pH 7.4). Incubation was carried out for 1 hr at 37° under 5% of CO_2. Catecholamines released into the medium and ^{22}Na taken up by the cells were measured. Data shown is one of the three experiments. The basal release of catecholamines and the influx of ^{22}Na in the absence of monensin were subtracted.

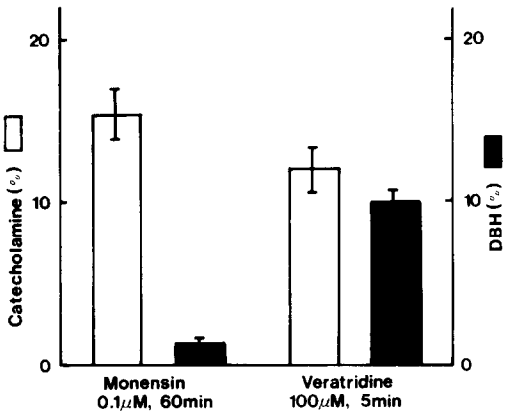

Fig. 3. Release of catecholamines and dopamine β-hydroxylase from cultured bovine adrenal medulla cells. Cultured bovine adrenal cells were incubated with monensin for 60 min or with veratridine for 5 min. Catecholamines and dopamine β-hydroxylase released into the medium were assayed. Release of catecholamines was expressed as the percentage to total catecholamines of the cells. Release of dopamine β-hydroxylase was expressed as the percentage to releasable dopamine β-hydroxylase activity of the cells. Values are the means of four experiments and standard deviations are indicated by the vertical bars.

monensin was not inhibited by 1 μM tetrodotoxin, while this toxin inhibited the influx of ^{22}Na caused by veratridine (Fig. 2). The release of catecholamines was also affected in a similar manner as the influx of ^{22}Na.

Release of catecholamines caused by monensin was not accompanied with the release of dopamine β-hydroxylase, while veratridine caused the co-release of this enzyme (Fig. 3).

Monensin caused the efflux of ^{45}Ca from the ^{45}Ca preloaded cells (Fig. 4). This effect of monensin did not require the Ca in the medium.

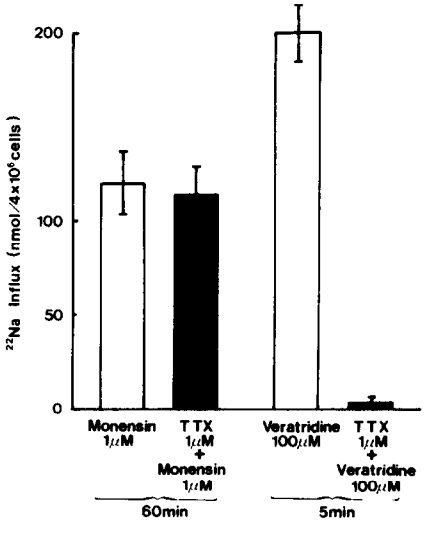

Fig. 2. Effect of tetrodotoxin on monensin and veratridine induced influx of ^{22}Na into cultured bovine adrenal medulla cells. Cultured bovine adrenal medulla cells were incubated with $^{22}NaCl$ in the presence of monensin for 60 min or in the presence of veratridine for 5 min. Tetrodotoxin 1 μM was present where indicated. Data shown are the means of four experiments and the standard deviations are indicated by the vertical bars.

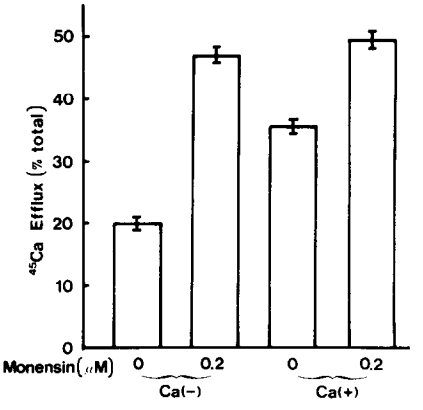

Fig. 4. Effect of monensin on the efflux of ^{45}Ca from ^{45}Ca preloaded cells. Cultured bovine adrenal medulla cells were preloaded with ^{45}Ca for 24 hr as reported elsewhere [8]. Before each experiment, cells were washed four times with Ca-free Krebs–Ringer phosphate buffer. Then cells were incubated for 1 hr in the presence and absence of monensin or Ca. Efflux of ^{45}Ca was expressed as the percentage to the ^{45}Ca preloaded to the cells. Values are the means of four experiments. Standard deviations are indicated by the vertical bars.

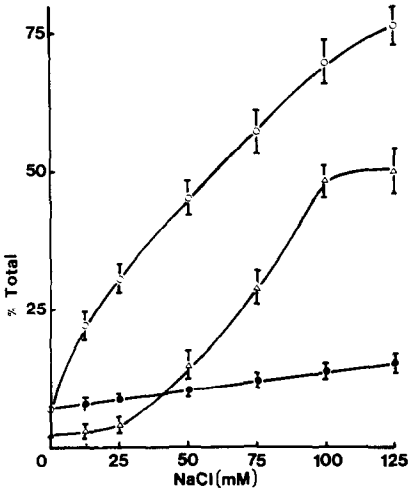

Fig. 5. Effect of monensin on the release of catecholamines and dopamine β-hydroxylase from the isolated chromaffin granules under various Na concentrations in the medium. Chromaffin granules (1500 μg of catecholamines, 2 mg of protein) were incubated with monensin (0.1 μM) under various Na concentrations in the medium. Tonicity of the medium was maintained by addition of appropriate concentrations of sucrose. Incubation was carried out for 15 min at 37°. Catecholamines and dopamine β-hydroxylase released into the medium were assayed and expressed by the percentage to total contents of the granules. Values are the means of four experiments and the standard deviations are indicated by the vertical bars (○—○: catecholamine release in the presence of monensin, ●—●: catecholamine release in the absence of monensin, △—△: dopamine β-hydroxylase in the presence of monensin).

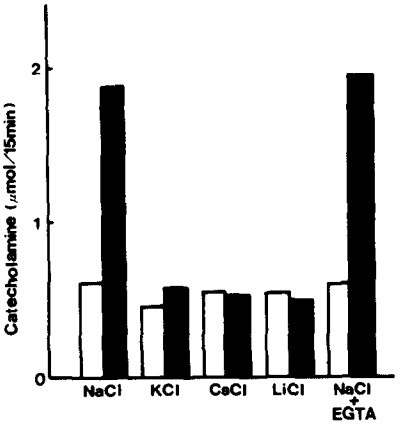

Fig. 6. Monensin-induced release of catecholamines from chromaffin granules in various mediums. Chromaffin granules were incubated in NaCl, KCl, CsCl, and in LiCl medium. The concentrations of these cations were adjusted to 25 mM and the tonicity was maintained by 225 mM sucrose. Incubation was carried out for 15 min at 37°. Monensin (0.1 μM) was present (shaded column) or absent (open column). Effect of EGTA (5 mM) was tested in NaCl medium. Data shown are the means from three measurements.

Effects of monensin on the isolated chromaffin granules

Monensin caused a Na dependent release of catecholamines from the isolated chromaffin granules. When Na concentration was below 25 mM, dopamine β-hydroxylase was not released into the medium, while this enzyme was also released by monensin at higher Na concentration (Fig. 5). When compared at the ionic strength of 25 mM, the effect of monensin was selectively dependent on Na. In K, Cs or Li medium, monensin did not cause the release of catecholamines (Fig. 6). Release of catecholamines by monensin was not diminished by removal of free Ca in the medium by addition of EGTA-[ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid].

Monensin caused the influx of ^{22}Na into the isolated chromaffin granules (Fig. 7). The concentration of monensin which causes the influx of ^{22}Na was slightly lower than that causing the release of catecholamines. Veratridine did not cause the release of catecholamines from the granules nor the influx of ^{22}Na to the granules (data not shown).

DISCUSSION

In the exocytotic release of catecholamines, Ca acts as the coupler between the stimulus and secretion [12]. Recently, we reported that influx of Na to the cells is a requisite for the influx of Ca and for the secretion of catecholamines evoked by acetylcholine and veratridine [3–5]. As has been reported already [1], monensin caused the release of catecholamines from cultured bovine adrenal medulla cells, which was dependent on Na but not on Ca in the medium. In our experiment, monensin caused the influx of ^{22}Na to the cells at the same concentration as it caused the release of catecholamines. This finding shows that elevation of intracellular

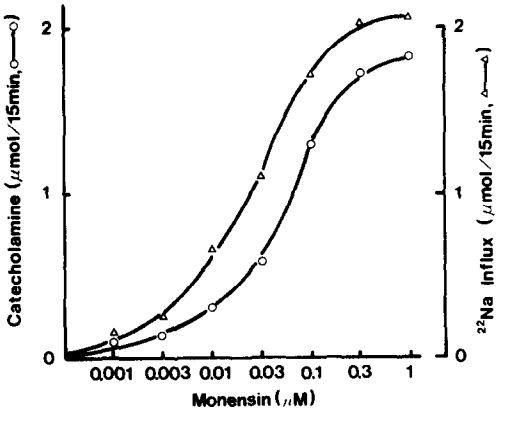

Fig. 7. Monensin-induced influx of ^{22}Na to the chromaffin granules and the release of catecholamines. Chromaffin granules were incubated with $^{22}NaCl$ in 25 mM NaCl–225 mM sucrose medium for 15 minutes at 37°. Catecholamines released into the medium and ^{22}Na taken up by the granules were measured. Data shown is one of the three experiments. The basal release of catecholamines and ^{22}Na influx were subtracted.

Na concentration is linked to the release of catecholamines.

It is interesting to compare the effect of monensin to that of veratridine, another Na ionophore which causes the release of catecholamines depending on Na and Ca in the medium. Influx of ^{22}Na caused by monensin was not inhibited by tetrodotoxin while the influx by veratridine was inhibited completely (Fig. 2). This finding shows that monensin causes the influx of Na by a mechanism different from veratridine. Monensin seems to act as mobile Na carrier across the membrane, while veratridine activates voltage-dependent Na channels. The influx of ^{22}Na caused by monensin was less than that caused by veratridine (Fig. 2), but the release of catecholamines was slightly greater (Fig. 3). This may indicate that elevation of Na concentration by monensin is more efficiently utilized to the release of catecholamines. This finding may also be related to the fact that monensin did not require Ca for its action, while veratridine requires Ca in addition to Na and Na acts by promoting the influx of Ca into the cells [4].

Another difference between monensin and veratridine is that monensin did not cause the release of dopamine β-hydroxylase from the cells, while veratridine did. This shows that monensin causes the release of catecholamines by a nonexocytotic manner.

Monensin caused the influx of ^{22}Na into the isolated chromaffin granules, simultaneously with the Na dependent release of catecholamines from the granules. Two mechanisms could be proposed to account for monensin induced release of catecholamines from the granules. Chromaffin granules have an acidic interior and enhancement of Na-proton exchange caused by monensin may dissipate the electrochemical proton gradient that is used as an energy source for accumulation of catecholamines as suggested by Johnson and Scarpa [13]. When electrochemical gradient is decreased, the stored catecholamines may diffuse out of the granules. This mechanism seems to contribute mainly when Na concentration in the medium is low (<25 mM), since dopamine β-hydroxylase was not released in low Na medium. The direct Na-catecholamine ion exchange mechanism [14] may also be involved in monensin-induced release of catecholamines. Alternatively, Na-proton exchange, evoked by monensin may cause the osmotic lysis of chromaffin granules and this mechanism seem to contribute much when Na concentration is high (>25 mM), since in high Na medium dopamine β-hydroxylase was released from the granules showing the lysis of granules.

It has been suggested that monensin causes the release of catecholamines by the mobilization of endogenous Ca through the elevation of Na concentration in the cells [1, 2]. We investigated the effect of monensin on the efflux of ^{45}Ca from ^{45}Ca preloaded cells and observed that monensin caused the efflux of ^{45}Ca (Fig. 4). However, the release of catecholamines from the chromaffin granules caused by monensin was not abolished by removal of Ca from the medium (Fig. 6). Therefore, although monensin actually causes the mobilization of endogenous Ca, it seems that the mobilized Ca is not necessarily required for the release of catecholamines by monensin.

Based on these findings in cultured adrenal medulla cells and isolated chromaffin granules, we conclude that monensin causes the release of catecholamines by acting as Na ionophore both at cell membranes and chromaffin granule. Release of catecholamines by monensin occurs by a nonexocytotic mechanism which is not dependent on Ca.

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