A Small Increment of External K Concentration Accelerates Adrenomedullary Catecholamine Secretion Induced by Ouabain and Metabolic Inhibitors

Masaru SORIMACHI and Shigeto NISHIMURA

*Department of Physiology, Kagoshima University, School of Medicine,
Kagoshima, Kagoshima, 890 Japan*

Summary In the cat adrenal perfused with a medium containing ouabain, raising the external concentration of K to 10 mM abruptly increased the rate of catecholamine secretion. Increased response to 10 mM K were also observed in the adrenals treated with monoiodoacetate or cyanide, which was suggested to reduce the Na pumping activity indirectly. Since both the onset time and the magnitude of K-induced secretion are dependent on the concentration of external Na, it was suggested that a small increase of K concentration accelerates Ca influx in exchange for internal Na.

Key Words: adrenal medulla, catecholamine, ouabain.

Available evidence suggests that the adrenal chromaffin cells possess a Na-Ca exchange system similar to that described in the squid axon and cardiac muscle (see review by BAKER and REUTER, 1975). The rate of ^{45}Ca efflux from the medullary slices (RINK, 1977) and the perfused cortex-free medulla (AGUIRRE *et al.*, 1977) was largely dependent on external Na. Conversely, all the procedures leading to a rise in the concentration of internal Na was found to increase Ca influx in exchange for internal Na (that is, Na-dependent Ca influx) thereby increasing catecholamine (CA) secretion (ESQUERRO *et al.*, 1980; GARCIA *et al.*, 1980, 1981b; NISHIMURA *et al.*, 1981; SORIMACHI *et al.*, 1981; SORIMACHI and YAMAGAMI, 1983). Recently, GARCIA *et al.* (1981a) reported that ouabain treatment of the adrenal gland enabled a small increment of external K to increase markedly the rate of CA secretion. Since this response persists in the presence of a low concentration of external Na (25 mM), a condition abolishing CA secretion due to Na-dependent Ca influx mechanism, they concluded that another component of the ouabain's effect was involved in this secretion. However, in contrast to their conclusion, we now present evidence showing that a small increase in

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反町 勝, 西村茂人

external K concentration somehow increases CA secretion due to Na-dependent Ca influx.

Adrenal glands of the cats were retrogradely perfused at room temperature (25–30°C) with modified Locke's medium containing NaCl (150 mM), KCl (5 mM) CaCl_2 (2 mM), MgCl_2 (1 mM), Tris/Cl (5 mM, pH 7.0–7.3), and glucose (5.5 mM) (DOUGLAS and RUBIN, 1961). Effluents were collected in test tubes containing appropriate amounts of acetic acid, so that final pH becomes about 4. Total CA was determined by the conventional fluorimetric method (ANTON and SAYRE, 1962).

Exposure of the adrenal to a medium containing ouabain (50 μM) caused a time-dependent increase of CA secretion. During this period, raising external K from 5 to 10 mM caused an abrupt increase of secretion rate and this rate immediately diminished following reduction of the concentration of K to 5 mM. These results are essentially similar to those observed by GARCIA *et al.* (1981a). However, in sharp contrast to their findings, we have found that the potentiating effect of 10 mM K was dependent on the concentration of Na in the medium containing ouabain when Na was partially replaced by Tris. As shown in Fig. 1, a 5 min exposure to 10 mM K increased secretion rate time-dependently during perfusion with a medium containing 50 mM Na (107 mM Tris) only in the presence of ouabain. When the concentration of Na was reduced to 20 mM (138 mM Tris), the significant increase of secretion rate in response to 10 mM K was observed only 45–70 min after introduction of ouabain depending on the experiments ($N=4$). Thereafter, the magnitude of the response to 10 mM K gradually increased; for example, a seventh exposure to 10 mM K at 15 min intervals released 2.9 and 8.0 μg CA during a 5 min period above the prestimulation level in two experiments.

On the other hand, when a low Na medium (25 mM) was prepared by substituting Na by choline instead of Tris, the substantial increase of secretion rate was observed in response to 10 mM K introduced at the 15th–20th min period, confirming the results by GARCIA *et al.* (1981a). However, of importance are our two findings that this response does not require the presence of ouabain, and that cholinergic antagonists (1.8 mM hexamethonium and 0.1 mM atropine) completely abolish this secretion. The recent finding of choline as a partial nicotinic agonist at the adrenal medulla (HOLZ and SENTER, 1981; SORIMACHI and NISHIMURA, 1982) raises the possibility that secretory effect of 10 mM K in the presence of choline is somehow related to the activation of acetylcholine (ACh) receptors. Indeed, 10 mM K increased the rate of secretion significantly during continuous exposure to a medium containing ACh (50 μM) or pilocarpine (1 mM), a muscarinic agonist, unless cholinergic antagonists were present. The exact mechanism of K-induced secretion in the presence of ACh or pilocarpine was not explored in the present study, but these results demonstrate that the observation by GARCIA *et al.* (1981a) could not be an indication against the involvement of Na-dependent Ca influx mechanism in the K secretory response. In a medium containing 25 mM Na

Fig. 1. Effect of raising external K concentration to 10 mM for 5 min during perfusion with a medium containing 50 mM Na (107 mM Tris) in the presence and absence of ouabain (50 μ M). Note that no secretory response to 10 mM K was observed in the absence of ouabain. Essentially similar results were obtained in the other four experiments.

(replaced by choline) and cholinergic antagonists, 10 mM K induced a significant increase of secretion only after a long treatment period with ouabain, as was shown in Tris-substituted medium. Thus, in the light of the Na dependency of ouabain's effect on K-induced secretion, it seems reasonable to consider that Na-dependent Ca influx is accelerated by increasing K concentration.

To test this possibility further, we have investigated the effect of 10 mM K on CA secretion observed during exposure to a medium containing a metabolic inhibitor, since inhibition of either glycolysis or oxidative phosphorylation was previously suggested to activate this Ca influx mechanism indirectly *via* the inhibition of Na pumping (SORIMACHI and YAMAGAMI, 1983). As shown in Fig. 2, CA secretion gradually increased during exposure to a medium containing cyanide (0.2 mM) or monoiodoacetate (MIA, 0.2 mM), as was observed in the case of ouabain. Raising the concentration of K to 10 mM for 5 min during this period further increased the rate of secretion and this rate diminished in the presence of 5 mM K. The stimulatory effect of K lasted even after removal of MIA, but not of cyanide, a finding which is consistent with the different reversibility of the effects of these agents on Na-dependent Ca influx mechanism (SORIMACHI and YAMAGAMI, 1983).

The rate of CA secretion during perfusion with a medium containing 50 mM Na(replaced by Tris) and ouabain was also increased by the addition of Rb (5 mM),

Fig. 2. Effect of raising external K concentration to 10 mM during perfusion with modified Locke's medium containing moniodoacetate (MIA, 0.2 mM) and KCN (0.2 mM). The second pulse of 10 mM K was still effective in increasing secretion after removal of MIA, while that failed to increase secretion after removal of KCN. Similar results were obtained in the other three experiments in each case.

but not of Cs (5 mM). The secretory effect of a higher concentration of Cs was weakest among alkali metal ions, and this was considered to be due to its weakest depolarizing effect (SORIMACHI, 1968). If this is feasible, then the result suggests that reduction in the electrochemical gradient for Na during depolarization by K(10 mM) or K(5 mM) plus Rb(5 mM) diminishes the external Na-dependent Ca efflux thereby resulting in an increase of Na-dependent Ca influx, as described in squid axon and cardiac muscle (BAKER and REUTER, 1975). Alternative explanation would be that Ca channel is somehow modified by an increase of K or Rb so that Ca influx is further increased, since internal Na-dependent Ca influx is inhibited by Ca channel blockers (NISHIMURA *et al.*, 1981; SORIMACHI and YAMAGAMI, 1983).

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