A Function of Extracellular Ca^<2+> in Controls on Cell Survival and Cell Death During Chicken Embryogenesis

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SUMMARY

This paper describes a function of extracellular calcium ion (Ca^{2+}) that may maintain normal embryonic development by means of controlling the dose level. Experimental results *in vivo* show that an excess of Ca^{2+} outside the cell administered to the chick embryo (after 24-30 hr of incubation) caused either the induction or inhibition of cell death, depending on the dose.

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INTRODUCTION

Up to now, only a few investigations concerning the role of extracellular Ca^{2+} on cell death have been performed [1]. In normal embryonic development, the cell death (called programmed cell death or apoptosis) occurs at specific times and sites [2-13]. This process is characterized by DNA degradation into fragments the size of one or more nucleosomes (DNA ladder) [14, 15] and by specific morphological changes including chromatin condensation and nuclear fragmentation [4, 8, 13].

In the present study, we have shown that excess extracellular Ca^{2+} administered to the chick embryo caused either the induction or inhibition of cell death, depending on the dose. This dual function of extracellular Ca^{2+} on cell survival and cell death during embryogenesis suggests that controlling the level of this factor may maintain normal embryonic development. During our studies we detected the above phenomena (chromatin condensation, nuclear fragmentation) by supravital staining (see Materials and Methods).

MATERIALS AND METHODS

Fertilized hen's eggs (Plymouth Rock), incubated at 38℃, were used throughout.

Staging of embryo development. The staging of embryo development was based on morphological characteristics, and was designated according to the Eguchi stage seriation (a revision of Hamburger-Hamilton's) [16, 17].

Extracellular ion modification. Various doses of K^{+} or Ca^{2+} dissolved in distilled water were administered to an embryo through a window made in the egg shell via the chorioallantoic membrane (0.2 ml per embryo) at 24 hr of incubation. The sealed egg was allowed to develop further until 30 hr of incubation.

Methods of counting dead cells. Dead cells were distinguished from living cells by supravital staining and/or histochemical (metachromatic) methods. For supravital staining, the entire removed embryos were incubated in 0.01% (w/w) Nile blue sulfate at 37℃ for 20 min, and washed twice with modified Ringer's saline solution [3]. Because most dead cells are phagocytozed by macrophages (or by neighboring cells), the number of phagocytozed cells (chromatin condensation, nuclear fragmentation) can be used as an index of cell death [18]. Counting the number of phagocytozed cells was performed on the photographs of stained embryos. Data were expressed as the percent of dead cells by comparing the photographs, (×400) of adjacent serial sections each stained with Nile blue and hematoxylin-eosin.

RESULTS AND DISCUSSION

Numbers of apoptotic cells as functions of the extracellular free cations are given in Figure 1. The x=100 concentration corresponds to the normal value of the extracellular cation concentration of the chicken ($[K^+]_o$: 5.63 mM, $[Ca^{2+}]_o$: 1.63 mM). The apoptotic cell numbers in neuroblasts increased with increasing doses of extracellular K^+ with a plateau at higher concentrations. On the other hand, the apoptotic cell numbers in neuroblasts increased with a peak at x=110 concentration, then decreased with increasing doses of extracellular Ca^{2+}. This was also observed in somitic cells to a lesser extent. No apparent changes in apoptotic cell numbers were observed in hemocytoblasts or epidermoblasts.

The increase in apoptotic cell numbers with a modification of extracellular K^+ might be caused by a large intracellular inflow of K^+, which could facilitate intracellular pathways leading to apoptosis. Increased intracellular Ca^{2+} could result from Ca^{2+} inflow from extracellular fluid through membrane potential-dependent Ca^{2+} channels (which might be activated by the K^+ inflow). However, to induce apoptosis, cells need an increase in intracellular Ca^{2+} of about a hundred-fold over the physiological, extracellular Ca^{2+} concentration [1], and hence, Ca^{2+} inflow might not be a candidate inducer of apoptosis. Nevertheless, the induction or inhibition of the apoptotic process in neuroblastic and somitic cells depending on the dose of extracellular Ca^{2+} (as shown in Figure 1) suggests that the role of extracellular cations in embryos is different from that in adult animals. Moreover, it is probable that the effect of extracellular Ca^{2+} on apoptosis differs between cell types.

To conclude, we found in this study that extracellular Ca^{2+} as a control on cell survival or cell death is feasible, and that such a cation could contribute to the delicate developmental regulation during apoptosis. To confirm our assumptions about extracellular cation activities, we need to measure the intracellular ion concentrations and membrane potential of the cells at various stages during apoptosis.

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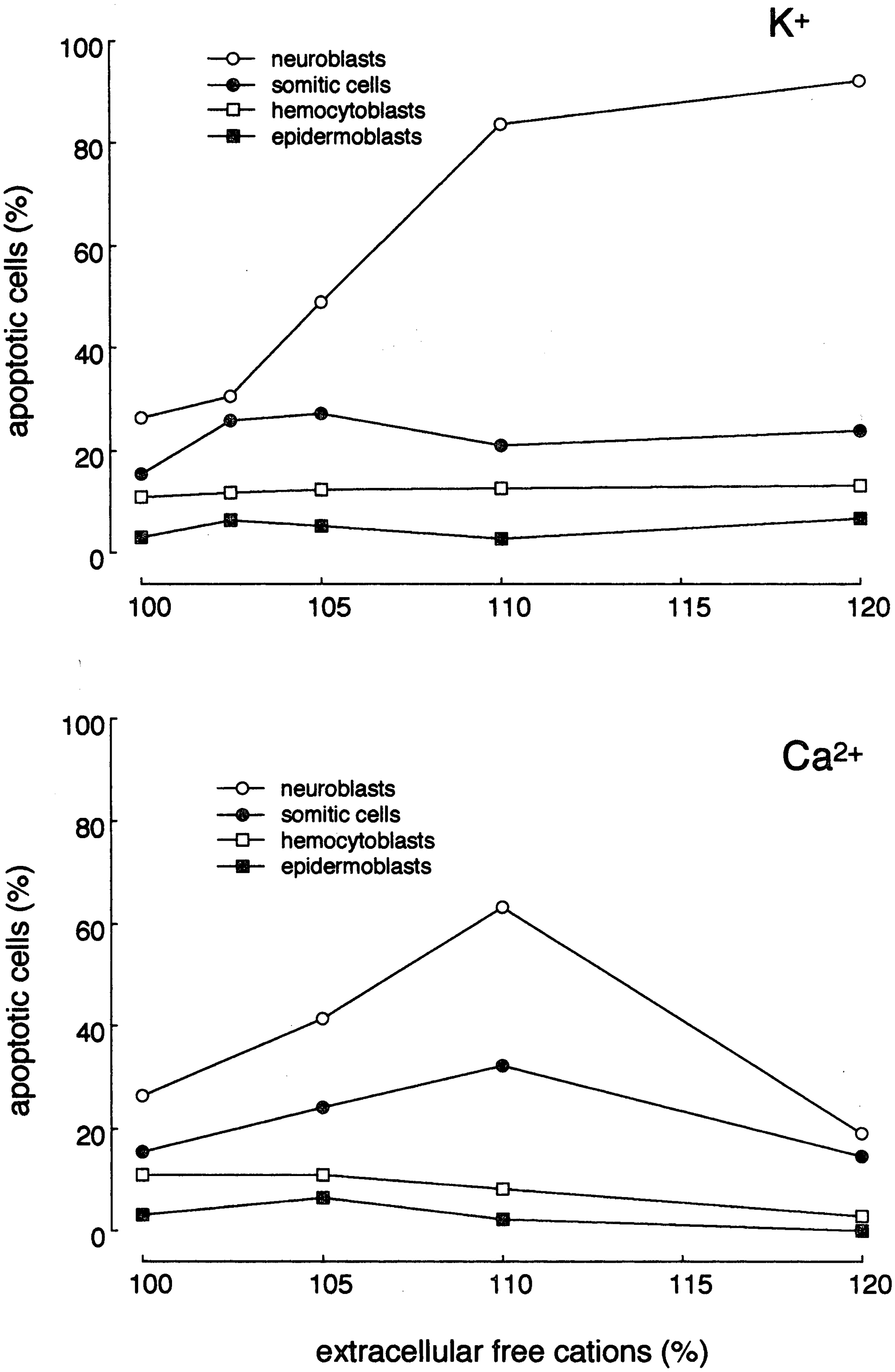

Fig.1. Apoptosis induced by excess extracellular cations (K^+, Ca^{2+}). Various doses of these cations were administered to a chick embryo at 24 hr of incubation. Dead cells were counted 6 hr later.

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胚発生過程の細胞生存および細胞死調節における細胞外カルシウムイオンの作用

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要　　約

本論文は細胞外カルシウムイオンのレベルが正常な胚発生に及ぼす作用について検討した。インビボにおける試験結果は、鶏胚に孵卵24時間目から30時間目の間に投与した細胞外のカルシウムイオンが、その投与量によって細胞死の誘導も阻害も引き起こすことを示した。

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