J. Membrane Biol. 16, 221–236 (1974)

Cyclic AMP Stimulation of Calcium Efflux from Kidney, Liver, and Heart Mitochondria

André B. Borle

Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. 15261

Received 5 October 1973

Summary. The effect of cyclic AMP on subcellular calcium turnover was studied in isolated kidney, liver and heart mitochondria. The calcium concentration of the incubating medium was determined by fluorometric methods after its separation by millipore filtration. Liver and kidney mitochondria take up calcium in exchange for H^+ and lower the medium calcium to 1 to 40×10^{-6} M in less than 2 min. Cyclic AMP produces an instantaneous release of calcium from mitochondria and a rise in the steady-state calcium concentration of the medium. A new medium calcium level of 0.7 to 3×10^{-4} M is achieved in less than 3 sec and is proportional to cyclic AMP concentrations between 10^{-7} and 3×10^{-6} M. Cyclic AMP is inactive above 5×10^{-6} M and below 10^{-7} M. Cyclic IMP, 5′AMP, dibutyryl cAMP are inactive at any concentration. Cyclic GMP is active at 10^{-5} M and competitively inhibits cyclic AMP action. The same steady-state calcium level is reached from higher or lower calcium concentrations, i.e. whether cyclic AMP is added before or after the addition of calcium to the mitochondrial suspension. At low calcium or phosphate concentrations, the calcium released by cyclic AMP is immediately reaccumulated by the mitochondria in less than 2 min with a further release of H^+. This "pulse" can be repeated by sequential additions of cyclic AMP. The transient or sustained response to cyclic AMP depends on the medium calcium × phosphate product and presumably on the presence or absence of calcium phosphate precipitate inside the mitochondria. These results support the hypothesis that cyclic AMP regulates cytoplasmic calcium by controlling the mitochondrial calcium efflux rate. This mechanism may be involved in the regulation of calcium transport and in some hormonal effects mediated by cyclic AMP.

Rasmussen has proposed that many cellular effects of cyclic 3′, 5′ adenosine monophosphate (cyclic AMP) may actually be due to a rise in cytoplasmic calcium concentration (Rasmussen, 1970; Rasmussen, Goodman & Tenenhouse, 1972). The source of this increased cytoplasmic calcium may be an increased influx of calcium from the extracellular fluids or a mobilization of calcium from intracellular stores. In support of this hypothesis, I have shown that parathyroid hormone (PTH) which increases the

cyclic AMP concentration in isolated kidney cells, stimulates cellular calcium influx (Borle, 1970, 1972*a*). Moreover, cyclic AMP between 10^{-9} and 10^{-7} M also increases calcium influx (Borle, *to be published*). I have further shown that both PTH and cyclic AMP increase calcium efflux from a subcellular compartment identified as the mitochondrial calcium pool (Borle, 1972*b*). If one compares the rates of calcium fluxes across the mitochondrial membrane with the calcium fluxes across the plasma membrane of the cell, it becomes evident that the cytoplasmic calcium activity must be influenced by the mitochondrial calcium turnover to a much greater extent than by calcium transport in and out of the cell (Borle, 1967, 1973*a*). In fact, the mitochondria may play the role of an intracellular calcium buffer system keeping the cytoplasmic calcium activity at very low levels (Borle 1967, 1971*a*, *b*; Lehninger, 1970). It is clear that the increased cellular calcium transport induced by PTH or cyclic AMP could be due to an increased cytoplasmic calcium secondary to a stimulation of the rate of calcium release from mitochondria (Borle, 1971*b*, 1973*a*). To test this hypothesis more directly, I have investigated the effects of cyclic AMP on calcium transport in isolated mitochondria. I have found that low concentrations of cyclic AMP produce an immediate release of calcium from isolated mitochondria and increase the extramitochondrial calcium concentration in direct proportion to the cyclic AMP levels (Borle, 1973*b*, *c*).

Materials and Methods

Mitochondria were prepared at 4 °C from adult Sprague Dawley rat liver, heart and kidney by standard methods. The animals were killed by decapitation. Tissues were homogenized in 0.25 M sucrose containing 10^{-4} M EDTA to prevent calcium accumulation by mitochondria during homogenization. The homogenate was centrifuged at $600 \times g$ for 15 min and the supernatant centrifuged at $14{,}000 \times g$ for another 15 min. The mitochondrial pellet was washed several times, resuspended in fresh sucrose containing no EDTA and centrifuged a second time at $14{,}000 \times g$ for 15 min. The reaction mixture consisted of equal parts of 0.25 M sucrose and of a KCl salt solution. Its final composition was: sucrose, 125 mM; Na ATP, 5 mM; Na succinate, 10 mM; $MgCl_2$, 4 mM; K_2HPO_4, 1 to 4 mM; Tris, 10 mM; KCl, 33 to 37 mM to a final osmolarity of 250 mosm. The initial pH of the suspending medium was 7.2.

The concentration of mitochondria in the reaction mixture was kept between 0.5 and 0.8 mg protein/ml suspension. Ten or 15-ml suspensions were incubated at 22 °C and constantly stirred with a magnetically driven Teflon stirrer. The pH was continuously monitored and recorded on a strip chart recorder. $CaCl_2$, cyclic AMP, and other nucleotides were added in volumes small enough (10 to 50 μliters or 0.1% to 0.5% of the suspension volume) not to significantly alter the initial conditions. One-ml aliquots of the suspension were taken at appropriate times with an Eppendorf pipette and the medium was immediately separated from the mitochondria by filtration through 0.45-μ millipore filters. The total medium calcium concentration was determined by fluorometric titration (Borle & Briggs, 1968).

Results

Liver Mitochondria

Fig. 1 shows that on addition of 500 μM $CaCl_2$ in a suspension mixture containing 4 mM phosphate, liver mitochondria accumulate the bulk of the added calcium in about 3 min and maintain a medium calcium concentration of 40 μM for as long as 15 min. In such conditions, the medium calcium will remain constant for at least 25 to 30 min. Longer incubation periods were not studied. Upon addition of 3×10^{-6} M cyclic AMP, the medium calcium rises five- to sixfold in less than 3 sec. Three seconds is the shortest interval tested between the addition of the nucleotide and the collection of the sample, allowing 2 sec for mixing. This higher level of calcium in the suspending medium can be maintained up to 25 min. When the same concentration of cyclic AMP is added at time zero, a few seconds before the addition of $CaCl_2$, the medium calcium concentration does not drop to control levels; it reaches the same elevated levels as those achieved by addition of cyclic

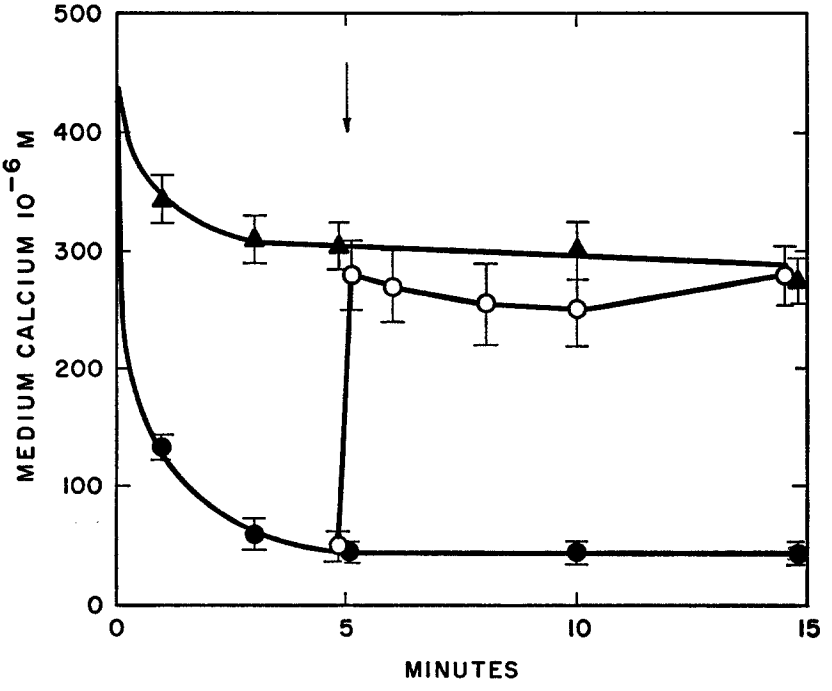

Fig. 1. Effect of 3×10^{-6} M cyclic AMP on calcium uptake by liver mitochondria. Experimental conditions: 0.5 to 0.8 mg protein/ml; total volume = 10 ml; ATP = 10 mM; succinate = 10 mM; Tris = 10 mM; sucrose = 125 mM; KCl to 250 mOsm; Mg = 4 mM; P_i = 4 mM; initial pH = 7.2; 5 μmoles $CaCl_2$ added at time zero; ●—● control (mean ± SE, n = 7); ▲—▲ 3×10^{-6} M cyclic AMP added at time zero (n = 5); ○—○ 3×10^{-6} M cyclic AMP added at 5 min (n = 6)

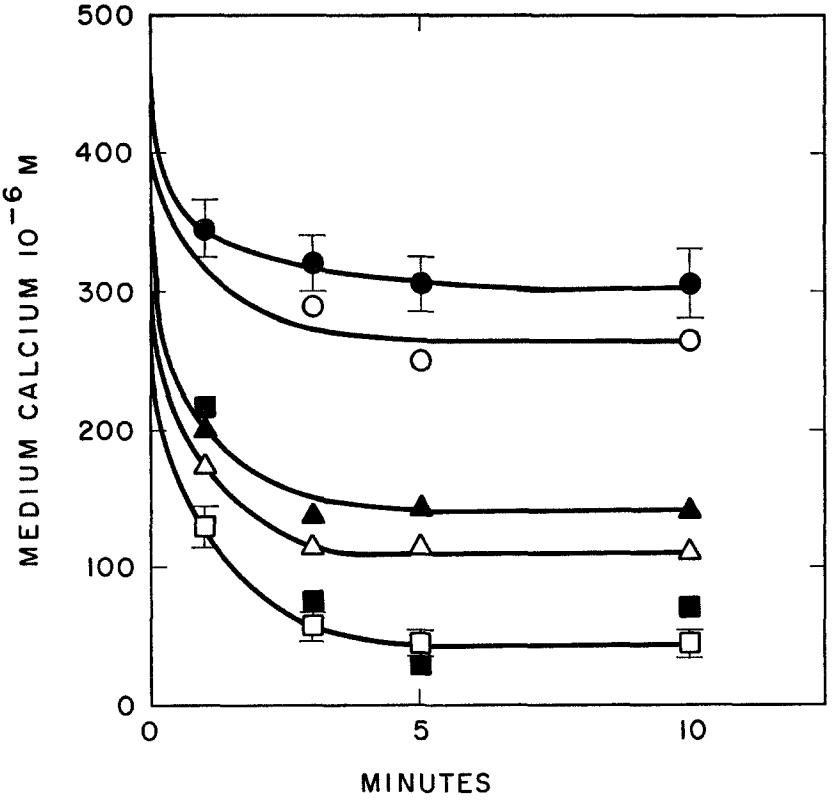

Fig. 2. Effect of cyclic AMP added before calcium at time zero on calcium uptake by liver mitochondria. Experimental conditions: same as in Fig. 1; □—□ control (mean ±SE, n=7); ■—■ cyclic AMP 10^{-7} M; △—△ cyclic AMP 5×10^{-7} M; ▲—▲ cyclic AMP 10^{-6} M; ○—○ cyclic AMP 2×10^{-6} M; ●—● cyclic AMP 3×10^{-6} M (n=5)

AMP at 5 min. The medium calcium concentrations achieved by addition of cyclic AMP at time zero or at 5 min are not statistically different. The level of calcium maintained in the suspending medium is proportional to the concentration of cyclic AMP, whether the nucleotide is added at time zero (Fig. 2) or whether it is added at 5 min (Fig. 3). Cyclic AMP activity decreases above 3×10^{-6} M and the nucleotide is inactive at concentrations greater than 5×10^{-6} M (Fig. 4).

Kidney Mitochondria

The effects of cyclic AMP on kidney mitochondria are similar to those obtained in liver mitochondria. Fig. 5 shows that the medium calcium concentration set by a given concentration of cyclic AMP can be reached

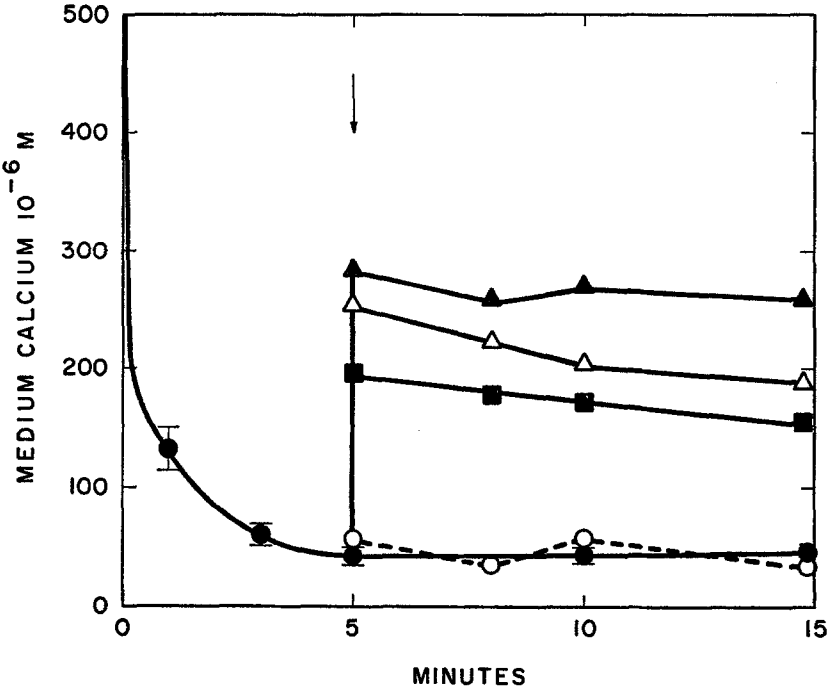

Fig. 3. Effect of cyclic AMP on the calcium concentration of liver mitochondria suspending medium. Experimental conditions: same as in Fig. 1: Cyclic AMP added at 5 min. ●—● control; ○—○ cyclic AMP 10^{-7} M; ■—■ cyclic AMP 5×10^{-7} M; △—△ cyclic AMP 10^{-6} M; ▲—▲ cyclic AMP 2×10^{-6} M

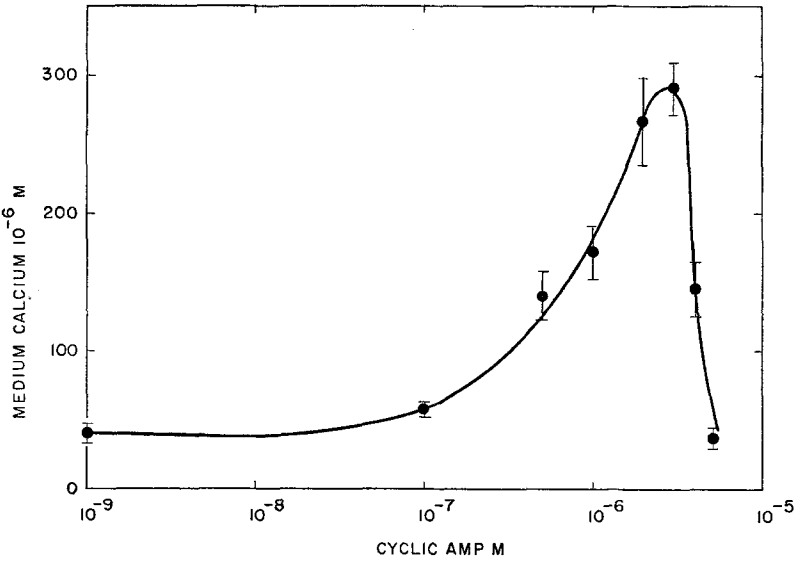

Fig. 4. Log-dose response curve of cyclic AMP on the calcium concentration of liver mitochondria suspending medium. Experimental conditions: same as in Fig. 1. Each point is the mean ±SE of 3 to 5 determinations

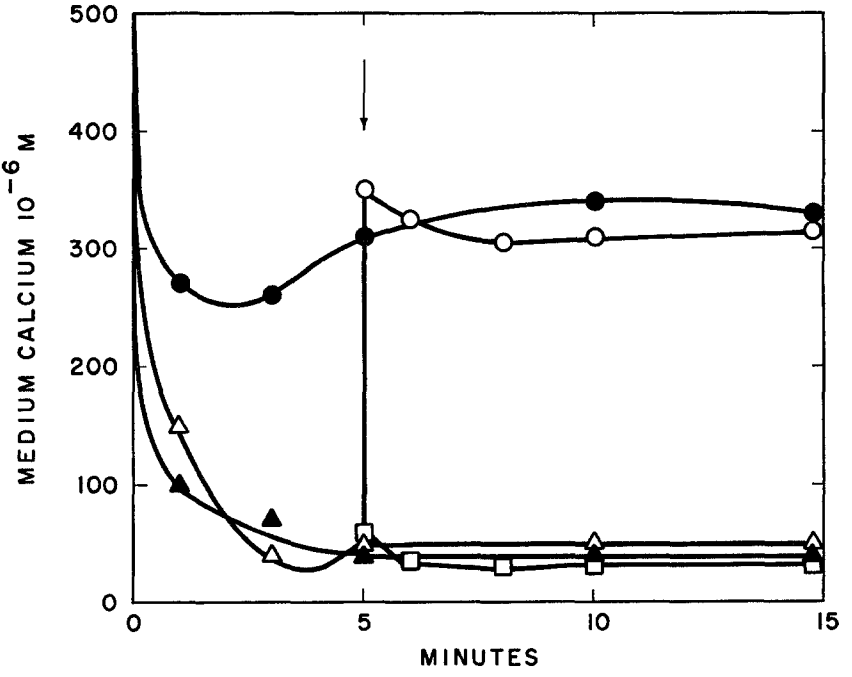

Fig. 5. Effect of cyclic AMP on kidney mitochondria. Experimental conditions: same as in Fig. 1. ▲—▲ Control; ●—● 3×10^{-6} M cyclic AMP added at time zero; ○—○ 3×10^{-6} cyclic AMP added at 5 min; △—△ 5×10^{-6} M cyclic AMP added at time zero; □—□ 5×10^{-6} M cyclic AMP added at 5 min

from higher or from lower calcium levels; the maximum effect is obtained with 3×10^{-6} M cyclic AMP; the nucleotide is inactive at concentrations of 5×10^{-6} M or above.

Heart Mitochondria

Fig. 6 shows that cyclic AMP produces the same response in heart mitochondria. The pH or the substrate utilized in these experiments may not be the optimal conditions for calcium uptake by heart mitochondria. Nevertheless, despite a slower calcium uptake, heart mitochondria readily respond to the nucleotide by releasing calcium and by maintaining a higher calcium concentration in the medium. In heart mitochondria, however, the maximum effect is obtained with 2×10^{-6} M cyclic AMP.

Effects of Calcium and Phosphate

In liver and in kidney mitochondria, decreasing the phosphate concentration from 4 to 1 mM changes the character of the response to cyclic

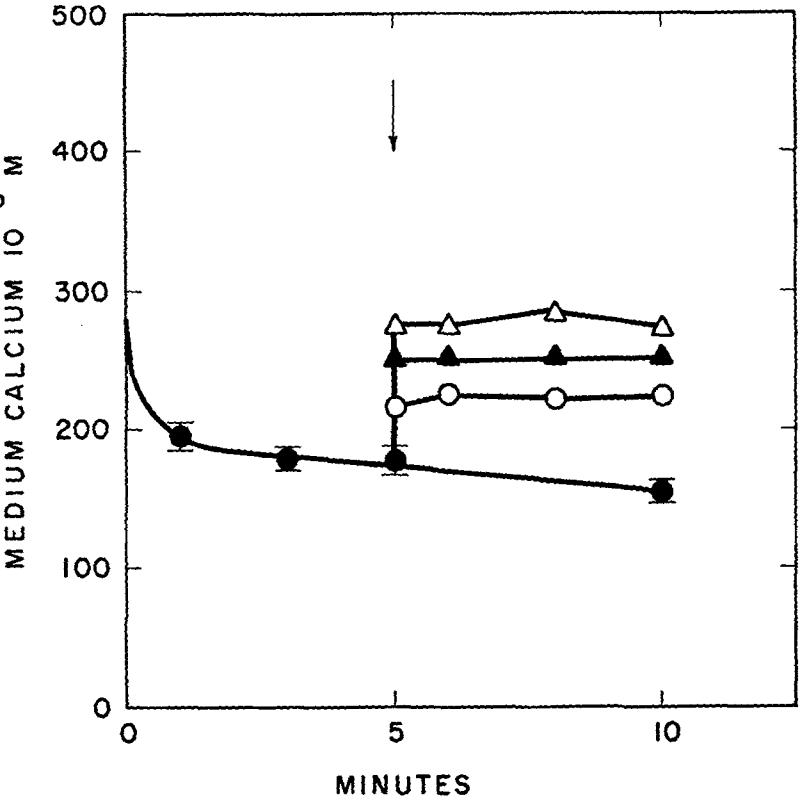

Fig. 6. Effect of cyclic AMP on heart mitochondria. Experimental conditions, same as in Fig. 1; 2.5 μmoles $CaCl_2$ added at time zero; cyclic AMP added at 5 min; •—• control; ○—○ 5×10^{-7} M cyclic AMP; ▲—▲ 10^{-6} M cyclic AMP; △—△ 2×10^{-6} M cyclic AMP

AMP, especially with small calcium loads. Fig. 7 shows that, after the addition of 500 μM $CaCl_2$ to liver mitochondria, the response to 2×10^{-6} M cyclic AMP is the same as with 4 mM phosphate. Decreasing the calcium load to 400 μM, however, depresses the steady-state calcium concentration triggered by cyclic AMP. Following calcium loads of 300 μM or lower, cyclic AMP only transiently increases the medium calcium which returns to control values in 1 to 5 min, depending on the load. The same phenomenon is observed in kidney mitochondria (Fig. 8). Thus, lowering the calcium or the phosphate concentration alters the pattern of response to cyclic AMP. At low calcium phosphate products, cyclic AMP triggers a pulselike release of calcium followed by an immediate reaccumulation by the mitochondria accompanied by a further release of H^+ ions (Fig. 9). This pulse of calcium release can be repeated several times (Fig. 10). Increasing again the phosphate concentration to 2 and 4 mM progressively shifts the pattern of response

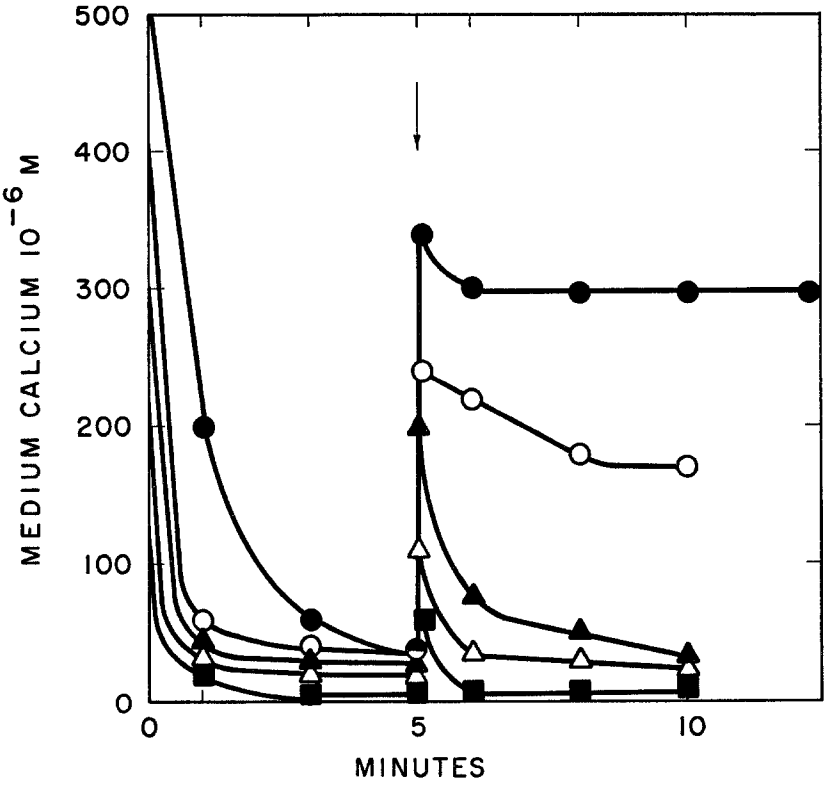
500
400
300
200
100
0
MEDIUM CALCIUM 10^{-6} M
0
5
10
MINUTES

Fig. 7

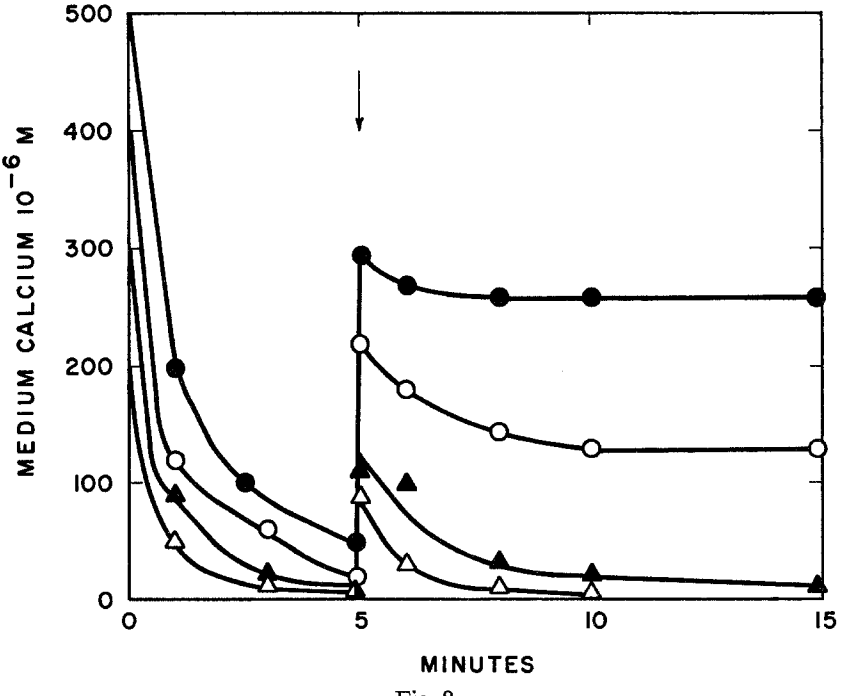
500
400
300
200
100
0
MEDIUM CALCIUM 10^{-6} M
0
5
10
15
MINUTES

Fig. 8

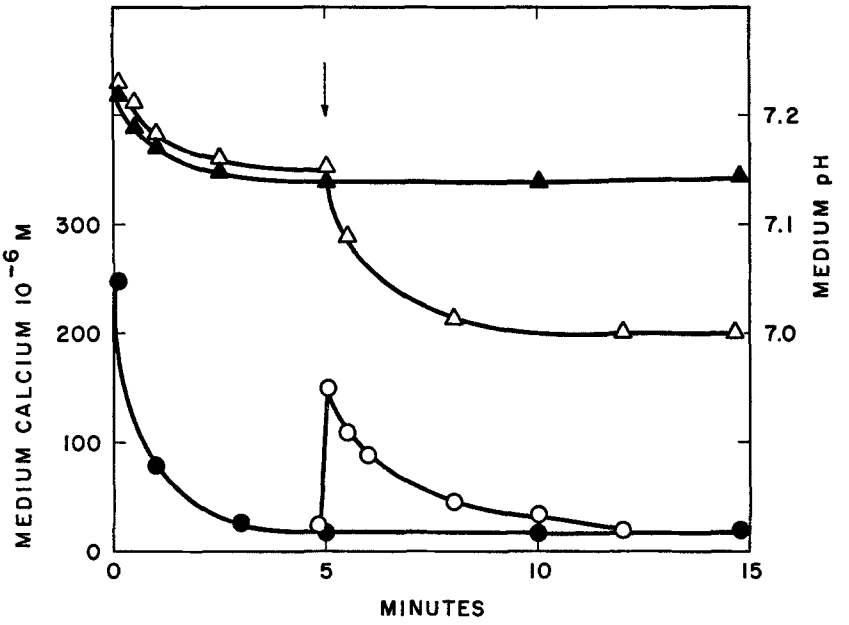

Fig. 9. Effect of cyclic AMP on kidney mitochondria in the presence of 1 mM phosphate. Experimental conditions same as in Fig. 7; 2.5 μmoles $CaCl_2$ added at time zero; 10^{-6} M cyclic AMP added at 5 min; •—• control medium calcium; ○—○ experimental medium calcium; ▲—▲ control medium pH; △—△ experimental medium pH

from a pulse to a sustained elevation of the medium calcium (Fig. 10). The same phenomenon can be obtained by maintaining the phosphate at 1 mM and progressively increasing the calcium load (Figs. 7 and 8).

Once the sustained pattern of response to cyclic AMP is achieved by higher phosphate concentrations or by increasing the calcium load, a further rise in phosphate concentration depresses the steady-state concentration of calcium in the medium. Fig. 11 shows that increasing the medium

Fig. 7. Effect of 2×10^{-6} M cyclic AMP on the calcium concentration of the suspending medium of liver mitochondria after varying loads of $CaCl_2$. Experimental conditions: same as in Fig. 1 except: $P_i = 1$ mM. One to 5 μmoles $CaCl_2$ added at time zero; 2×10^{-6}M cyclic AMP added at 5 min; ■—■ 1 μmole $CaCl_2$; △—△ 2 μmoles $CaCl_2$; ▲—▲ 3 μmoles $CaCl_2$; ○—○ 4 μmoles $CaCl_2$; •—• 5 μmoles $CaCl_2$

Fig. 8. Effect of 2×10^{-6} M cyclic AMP on the calcium concentration of the suspending medium of kidney mitochondria after varying loads of $CaCl_2$. Experimental conditions, same as in Fig. 7; $CaCl_2$ added at time zero; 2×10^{-6} cyclic AMP added at 5 min; △—△ 2 μmoles $CaCl_2$; ▲—▲ 3 μmoles $CaCl_2$; ○—○ 4 μmoles $CaCl_2$; •—• 5 μmoles $CaCl_2$

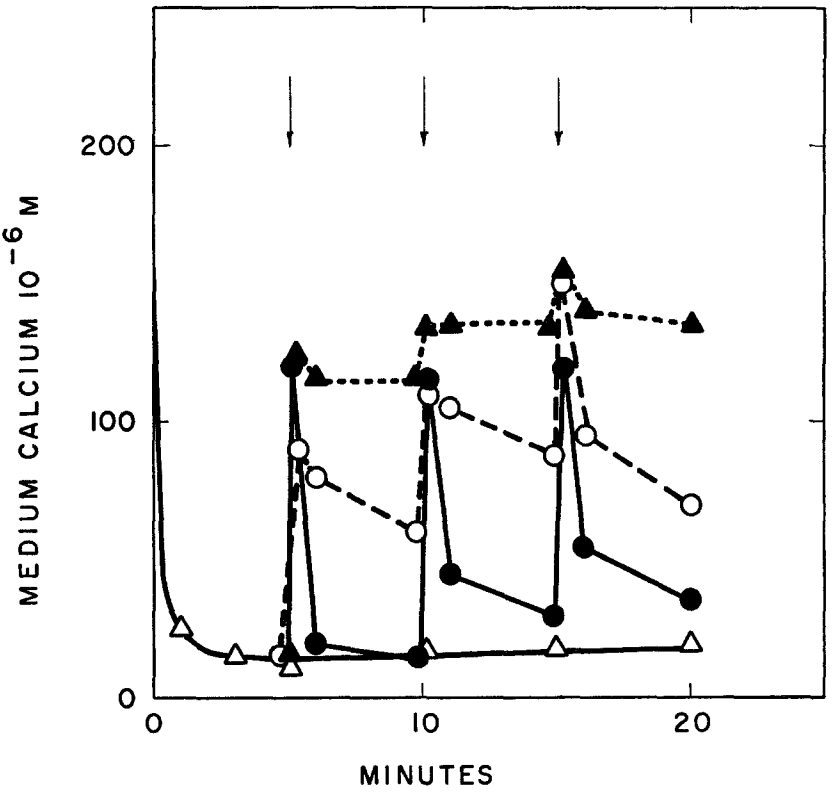

Fig. 10. Effect of repeated additions of 3×10^{-6} M cyclic AMP to kidney mitochondrial suspensions with varying phosphate concentrations. Experimental conditions: same as in Fig. 1 except: total volume = 15 ml; $P_i = 1$ to 4 mM; 1.5 μmoles $CaCl_2$ added at time zero; 3×10^{-6} M cyclic AMP added at 5, 10 and 15 min (arrows). △—△ control, 1 mM P_i; ●—● cyclic AMP, 1 mM P_i; ○ – – ○ cyclic AMP, 2 mM P_i; ▲---▲ cyclic AMP, 4 mM P_i

phosphate concentration from 1 to 7 mM immediately depresses the medium calcium down to control levels. A more progressive rise in phosphate from 1 to 2 mM and from 2 to 3 mM produces a stepwise reduction of the medium calcium concentration.

Effects of Other Nucleotides

Other nucleotides such as dibutyryl cyclic AMP, 5′ AMP, ATP and cyclic IMP had no effect at any concentration ranging from 10^{-8} to 10^{-3} M. However, preliminary experiments revealed that cyclic GMP had a slight effect at an optimal concentration of 10^{-5} M. The maximum effect obtained with cyclic GMP is less than with optimal concentrations of cyclic AMP. After cyclic GMP addition rise in medium calcium concentration is only

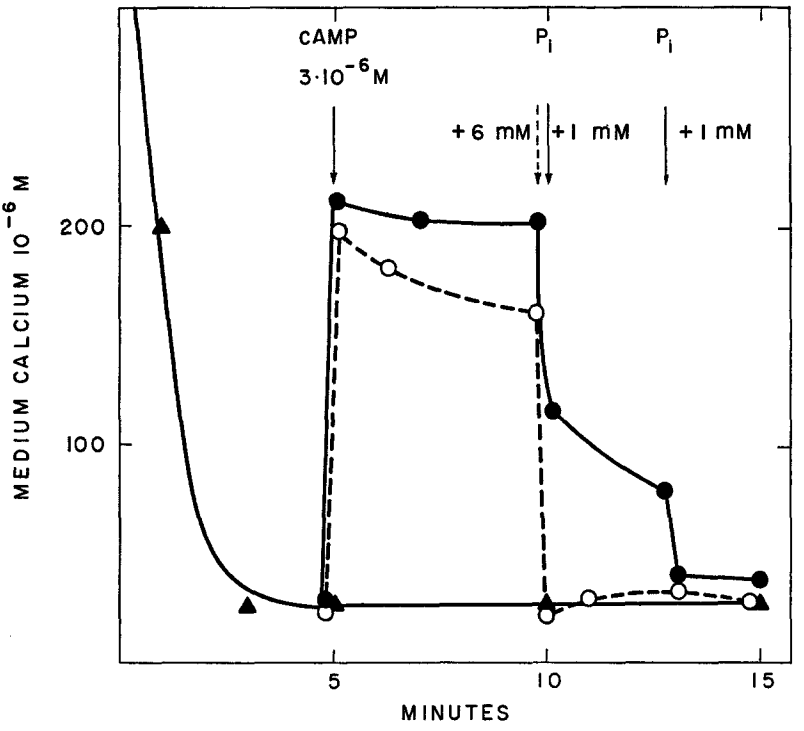

Fig. 11. Effect of increasing the medium phosphate concentration on the steady-state calcium level set by 3×10^{-6} M cyclic AMP. Experimental conditions: same as in Fig. 1 except: total volume = 15 ml; initial $P_i = 1$ mM; 7.5 μmoles of $CaCl_2$ added to the 15-ml suspension at time zero; 3×10^{-6} M cyclic AMP added at 5 min; o — — o medium P_i concentration increased to 7 mM at 10 min; •—• medium P_i concentration increased to 2 mM at 10 min and to 3 mM at 13 min. ▲—▲ control

transient; it reaches a maximum within 2 to 3 min in contrast to cyclic AMP which acts within seconds; the effect decays in about 5 min. Increasing concentrations of cyclic GMP progressively depress the effect of cyclic AMP suggesting competitive inhibition between the two cyclic nucleotides. Work is presently in progress to elucidate the nature of the interactions between these and other nucleotides.

Discussion

These results suggest that both cyclic AMP and mitochondria play an important role in cellular calcium homeostasis. The response of mitochondria to cyclic AMP may be characterized as follows: (1) The response is immediate; it takes less than a few seconds for the medium calcium to rise almost

10-fold. This is due to the rapidity of the calcium exchange taking place between the mitochondrial matrix and the surrounding fluids: with extremely high rate of calcium influx and efflux, any change in one of the rate constants will allow a new steady state to be reached almost instantaneously. (2) The response is dose dependent between the cyclic AMP concentrations of 10^{-7} and 3×10^{-6} M. Higher concentrations of the nucleotide are progressively less effective and concentrations greater than 5×10^{-6} M are totally ineffective. This bell-shaped pattern of response to cyclic AMP is not unusual, although totally unexplained (Kuo & Greengard, 1969; Rixon, Whitfield & MacManus, 1970; MacManus, Perris, Whitfield & Rixon, 1970; Klein & Raisz, 1971). (3) The response can be either sustained or transient, depending on the calcium and phosphate concentration of the suspending medium. The transient response observed with low concentrations of phosphate can be progressively transformed to a sustained one if the calcium concentration is increased (Figs. 7 and 8). Conversely, the transient pattern observed with low concentrations of calcium shifts to a sustained response if the phosphate concentration is increased (Fig. 10). The type of response is apparently related to the calcium phosphate product. It may depend upon the presence or absence of calcium phosphate precipitate in the mitochondrial matrix. A possible explanation could be that, in the presence of calcium phosphate precipitate in the mitochondrial matrix, the calcium activity in the mitochondria would be fixed by the solubility product of the particular calcium salt. In these conditions, a change in the rate constant of calcium efflux would be immediately reflected by a sustained change in the medium calcium concentration.

The rise in medium calcium produced by cyclic AMP could be due to an increased calcium efflux from the mitochondria or to an inhibition of calcium uptake. The most likely cause is a sudden increase in calcium efflux for the following reasons: (1) Even in the presence of cyclic AMP the mitochondria are able to take up calcium and to restore the medium calcium concentration to its original low levels, when the calcium or the phosphate concentrations of the medium are low. (2) The rate of calcium uptake by mitochondria following cyclic AMP-induced calcium release is as rapid as the initial accumulation in the absence of the nucleotide. (3) The reaccumulation of calcium by the mitochondria after cyclic AMP is accompanied by an additional and expected evolvement of H^+ ions into the medium. Consequently, one can conclude that cyclic AMP stimulates the rate constant of calcium efflux from mitochondria. As I have proposed earlier (Borle, 1973*a*) the steady-state exchange of calcium between the mitochondria and their environment can be described as follows: at steady

state, calcium influx from medium to mitochondria J_{cm} must be equal to the efflux of calcium from mitochondria to the medium J_{mc}. Calcium uptake by mitochondria must follow the general equation:

$$J_{cm} = k_{cm} \cdot \mathrm{Ca}_c \tag{1}$$

where k_{cm} is the rate constant of calcium influx into mitochondria and Ca_c, the calcium activity of the medium. Calcium efflux from mitochondria becomes:

$$J_{mc} = k_{mc} \cdot \mathrm{Ca}_m \tag{2}$$

where k_{mc} is the rate constant of calcium efflux from mitochondria and Ca_m the calcium activity in the mitochondria. At steady state,

$$J_{cm} = J_{mc} \tag{3}$$

therefore

$$k_{cm} \cdot \mathrm{Ca}_c = k_{mc} \cdot \mathrm{Ca}_m \tag{4}$$

or

$$\mathrm{Ca}_c = \frac{\mathrm{Ca}_m \cdot k_{mc}}{k_{cm}}. \tag{5}$$

According to Eq. (5), it is obvious that if the mitochondrial calcium activity Ca_m is constant, fixed by the solubility product of a calcium phosphate precipitate, an increase in the rate constant of efflux, k_{mc}, triggered by cyclic AMP will immediately produce a sustained rise in the medium, Ca_c. On the other hand, if the calcium phosphate product of the medium is too low to allow the precipitation of calcium phosphate in the mitochondrial matrix, Ca_m will not be constant. In these conditions, the rise in k_{mc}, induced by cyclic AMP, will transiently increase the medium calcium Ca_c. However, the new steady state will be achieved with a decreased mitochondrial calcium activity Ca_m, totally compensating the rise in k_{mc} so that the medium calcium Ca_c will return to control levels. Such a behavior has been confirmed by computer simulation of the system (A. B. Borle and J. Anderson, *to be published*).

The identical pattern of response to cyclic AMP by kidney, liver and heart mitochondria suggests that this mechanism of action may be fundamental and an integral part of the cell second messenger signal system. These results confirm previous findings obtained in isolated cells showing that cyclic AMP stimulates calcium efflux from the mitochondrial compartment and secondarily increases cytoplasmic calcium (Borle, 1972). They support earlier proposals that cyclic AMP may act by increasing the cyto-

plasmic activity (Rasmussen, 1970; Rasmussen *et al.*, 1972; Borle, 1971*b*, 1972*a*, 1973*a*). According to the present studies, the source of the calcium appears to be the mitochondria rather than the extracellular fluids. The fact that isolated mitochondria can instantaneously regulate the calcium concentration of their surrounding fluids in precise proportion to the cyclic AMP concentration provides an ideal control mechanism for the physiological regulation of many cellular functions. It suggests that, in the cell, mitochondria may serve as the controller and the regulator of cytoplasmic calcium.

In cells involved in calcium transport, the sustained increase in cytoplasmic calcium would result in an increased calcium efflux from the cell because of the increased substrate presented to the calcium pump mechanism. Calcium influx, which is presumed to be passive, may also be increased since a rise in cytoplasmic calcium has been shown to increase the membrane permeability to ions and other uncharged solutes (Lew, 1970; Godfraind, Kawamura, Krnjevic & Pumain, 1971; Romero & Whittam, 1971; Baker, 1972; Krnjevic & Lisiewicz, 1972). And indeed, increased calcium influx and efflux have been observed in isolated kidney cells after cyclic AMP or parathyroid hormone administration (Borle 1970, 1972*a*). In liver and other cells, an increased cytoplasmic calcium may produce many metabolic effects usually attributed to cyclic AMP and may actually play the role of an intracellular messenger as suggested by Rasmussen (1970, 1972).

Finally, the effects of cyclic AMP obtained in isolated heart mitochondria offer an alternative to the many explanations offered for the inotropic effects of catecholamines and of cyclic AMP (Sobel & Mayer, 1973). A sustained elevation of the cytoplasmic calcium activity of heart cells, produced by the action of cyclic AMP on the mitochondrial calcium turnover, could explain the increased contractility of cardiac muscle induced by catecholamines. This "tonic" influence of mitochondria on the sarcoplasmic calcium activity could complement the "phasic" regulation provided by the sarcoplasmic reticulum.

It is clear that more work needs to be done to define the interactions between the cyclic nucleotides and mitochondria. It remains to be seen whether both ATP and succinate are necessary for these effects of cyclic AMP, and whether other substrates can be substituted. We already know that Na can be substituted for K without affecting the results. It is also evident that the calcium concentrations used are much higher than those prevailing in the intact cell. The experimental conditions were chosen for an optimal calcium uptake and retention by mitochondria. However, calcium loads of less than 50 μM have been tested allowing the mitochondria

to lower the medium calcium to 1 μM. In these conditions the calcium accumulated is completely released by the mitochondria when stimulated by cyclic AMP. More sophisticated techniques will be necessary to study calcium uptake and release with smaller calcium concentrations.

The technical assistance of Mrs. Marianne Biddle and Mrs. Jo Anne Bast is gratefully acknowledged. This work was supported by USPHS Grant No. AM 07867 from the National Institutes of Health.

References

Baker, P. F. 1972. Transport and metabolism of calcium ions in nerve. *In:* Progress in Biophysics and Molecular Biology. J. A. V. Butler and D. Noble, editors. Vol. 24, p. 179. Pergamon Press, New York

Borle, A. B. 1967. Membrane transfer of calcium. *Clin. Orthop.* **52**:267

Borle, A. B. 1970. Kinetic analyses of calcium movements in cell cultures. III. Effects of calcium and parathyroid hormone in kidney cells. *J. Gen. Physiol.* **55**:163

Borle, A. B. 1971*a*. Calcium transport in kidney cells and its regulation. *In:* Cellular Mechanisms for Calcium Transfer and Homeostasis. G. Nichols, Jr. and R. H. Wasserman, editors. p. 131. Academic Press Inc., New York

Borle, A. B. 1971*b*. Le turn-over du calcium dans l'organisme et les principaux points d'impact hormonaux. *In:* Les Hormones et le Calcium. H. P. Klotz, editor. p. 5. Expansion Scientifique Francaise, Paris

Borle, A. B. 1972*a*. Parathyroid hormone and cell calcium. *In:* Calcium, Parathyroid Hormone and the Calcitonins. R. V. Talmage and P. L. Munson, editors. p. 484. Excerpta Medica, Amsterdam

Borle, A. B. 1972*b*. Kinetic analysis of calcium movements in cell cultures. V. Intracellular calcium distribution in kidney cells. *J. Membrane Biol.* **10**:45

Borle, A. B. 1973*a*. Calcium metabolism at the cellular level. *Fed. Proc.* **32**:1944

Borle, A. B. 1973*b*. Cyclic AMP regulation of calcium efflux from liver, kidney and heart mitochondria. *J. Int. Res. Commun.* **1**:9

Borle, A. B. 1973*c*. Effects of cyclic AMP (cAMP) on Ca transport in mitochondria. *The Physiologist* **16**:270

Borle, A. B., Briggs, F. N. 1968. Microdetermination of calcium in biological material by automatic fluorometric titration. *Analyt. Chem.* **40**:339

Godfraind, J. M., Kawamura, H., Krnjevic, K., Pumain, R. 1971. Action of dinitrophenol and some other metabolic inhibitors on cortical neurones. *J. Physiol. (London)* **312**:199

Klein, D. C., Raisz, L. G. 1971. Role of adenine 3′,5′ monophosphate in the hormonal regulation of bone resorption: Studies with cultured fetal bone. *Endocrinology* **89**:818

Krnjevic, K., Lisiewicz, A. 1972. Injections of calcium ions into spinal motoneurons. *J. Physiol. (London)* **225**:363

Kuo, J. F., Greengard, P. 1969. Cyclic nucleotide-dependent kinases. IV. Widespread occurence of adenosine 3′5′-monophosphate-dependent protein kinase in various tissues and phyla of the animal kingdom. *Proc. Nat. Acad. Sci.* **64**:1349

Lehninger, A. L. 1970. Mitochondria and calcium transport. *Biochem. J.* **119**:129

Lew, V. L. 1970. Effect of intracellular calcium on the potassium permeability of human red cells. *J. Physiol. (London)* **206**:35P

MacManus, J. P., Perris, A. D., Whitfield, J. F., Rixon, R. H. 1970. Control of cell division in thymic lymphocytes by parathyroid hormone, thyrocalcitonin and cyclic adenosine 3′5′-monophosphate. *In: Proc. Fifth Leucocyte Culture Conf.* J. E. Harris, editor. p. 125. Academic Press Inc., New York

Rasmussen, H. 1970. Cell communication, calcium ion and cyclic adenosine monophosphate. *Science* **170**:404

Rasmussen, H., Goodman, D. B. P., Tenenhouse, A. 1972. The role of cyclic AMP and calcium in cell activation. *CRC Crit. Rev. Biochem.* **1**:95

Rixon, R. H., Whitfield, J. F., MacManus, J. P. 1970. Stimulation of mitotic activity in rat bone marrow and thymus by exogenous adenosine 3′,5′-monophosphate (cyclic AMP). *Exp. Cell Res.* **63**:110

Romero, P. J., Whittam, R. 1971. The control by internal calcium of membrane permeability to sodium and potassium. *J. Physiol.* (*London*) **214**:481

Sobel, B. E., Mayer, S. E. 1973. Brief review: Cyclic adenosine monophosphate and cardiac contractility. *Circ. Res.* **32**:407