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# COMPARISON OF THE CHANGES IN CYTOPLASMIC FREE CALCIUM CONCENTRATION INDUCED BY PHENYLEPHRINE, VASOPRESSIN AND ANGIOTENSIN II IN HEPATOCYTES

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Effects of phenylephrine, vasopressin and angiotensin II on cytoplasmic free calcium concentration, $[Ca^{2+}]_c$, were examined by monitoring aequorin bioluminescence in isolated hepatocytes preloaded with aequorin. In the presence of 0.5 mM calcium in the medium, the pattern of changes in aequorin bioluminescence induced by phenylephrine was different from that induced by vasopressin or angiotensin II. When extracellular calcium concentration was reduced to 1 $\mu$M, however, these three agents induced identical changes in aequorin bioluminescence. These results suggest that the mode of action of phenylephrine on cytoplasmic free calcium concentration differs from that of either vasopressin or angiotensin II and that the difference in ability to increase calcium influx may account for the distinct patterns induced by these agents. 

It is well known that $\alpha$-adrenergic agonists, vasopressin and angiotensin II increase hepatic glucose production and this effect is thought to be associated with increased cytoplasmic free calcium concentration, $[Ca^{2+}]_c$ (1-3). The mechanisms by which these agonists raise $[Ca^{2+}]_c$ are not fully understood. Since these agonists increase inositol 1,4,5-trisphosphate (4-7), it has been suggested that inositol 1,4,5-trisphosphate, which can mobilize calcium from an intracellular pool(s), may be a

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mediator to increase $[Ca^{2+}]_c$. Nonetheless, recent studies have suggested that modes of action of these three agents on cellular calcium metabolism may not be the same. Thus, increases in calcium influx induced by vasopressin and angiotensin II are greater (8) and increases in calcium efflux by vasopressin and angiotensin II are smaller than those by $\alpha$-adrenergic agonists (9). These results indicate that the mode of action of $\alpha$-adrenergic agonist on cellular calcium metabolism is different from those of vasopressin and angiotensin II. When effects of these three agents on $[Ca^{2+}]_c$ are measured by the use of quin-2, they cause identical changes in quin-2 fluorescence (3). Given the facts that quin-2 has considerable calcium chelating activity and that quin-2 buffers a subtle change in $[Ca^{2+}]_c$, (10), it is possible that quin-2 may be unable to discriminate the effects of these three agonists on $[Ca^{2+}]_c$. To examine this possibility, we compared the actions of phenylephrine, vasopressin and angiotensin II on $[Ca^{2+}]_c$, using a calcium sensitive photoprotein, aequorin. Results in the present study demonstrate that the effect of phenylephrine on $[Ca^{2+}]_c$ are distinct, in part, from those of vasopressin and angiotensin II.

## Materials and Methods

Parenchymal liver cells from fed male Wistar rats weighing 190-220g were isolated according to the methods described by Berry and Friend (11, 12). Isolated hepatocytes were incubated in modified Hanks solution containing 0.5 mM calcium (10). When the extracellular calcium concentration was fixed at 1 uM, Ca-EGTA buffer was used (8). Isolated hepatocytes were loaded with aequorin by the method of Morgan and Morgan (10) and bioluminescence was measured by Chrono-log platelet aggregometer (Havertown, PA, USA) under constant stirring at 37°C (10). Resting $[Ca^{2+}]_c$ was calibrated assuming the even distribution of calcium in the cell (10). $[Ca^{2+}]_c$ in stimulated cells was not calibrated since no information was available as to the spatial distribution of calcium in the cell. The magnitude of aequorin luminescence is expressed as electric current. Tracings presented in the Results are the representatives of at least five experiments with similar results. Glucose output in aequorin-loaded cells in response to phenylephrine was identical to that in unloaded cells (data not shown). Aequorin was purchased from Dr. J.R. Blinks of Mayo Foundation (Rochester, MN, U.S.A. ).

## Results

Fig. 1 shows the effects of phenylephrine, vasopressin and angiotensin II on $[Ca^{2+}]_c$ as assessed by aequorin luminescence in the medium containing 0.5 mM calcium. Resting $[Ca^{2+}]_c$ of the hepatocytes was 178 ± 22 nM (mean ± S.E, n=8). As shown in Fig. 1A, aequorin luminescence in responese to $10^{-5}$ M phenylephrine was characterized by an immediate sharp peak with a duration of 20 sec, which was followed by a second peak of less magnitude than the first peak, which lasted for about 60 sec. In response to smaller doses of phenylephrine, similar changes in luminescence were observed, but with smaller magnitude. Changes in aequorin luminescence in response

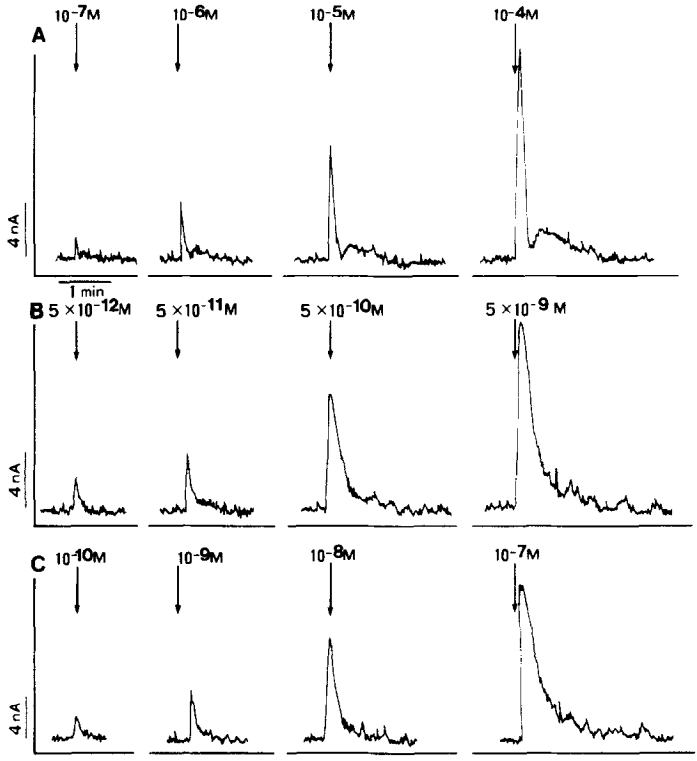


Fig. 1. Changes in $[Ca^{2+}]_c$ in response to phenylephrine, vasopressin and angiotensin II in aequorin-loaded hepatocytes incubated in medium containing 0.5 mM calcium.

Hepatocytes were stimulated by varing doses of phenylephrine ( A ), vasopressin ( B ) and angiotensin II ( C ) in the presence of 0.5 mM extracellular calcium. Each agent was added as indicated by the arrow.

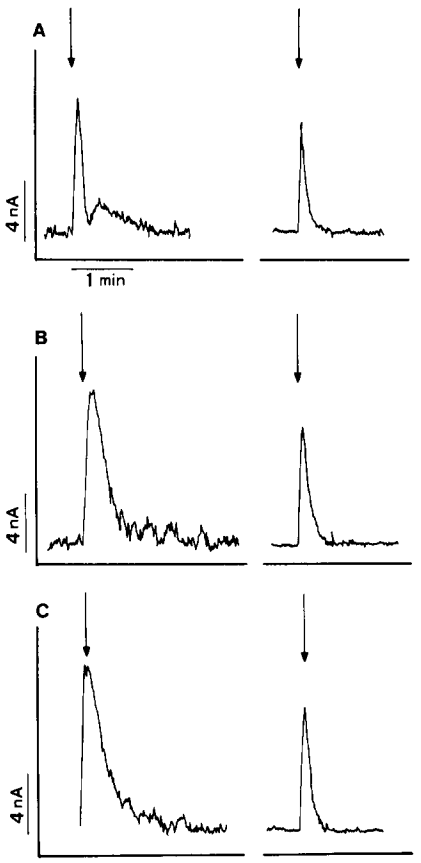


Fig. 2. Effect of extracellular calcium on phenylephrine-, vasopressin- and angiotensin II-induced changes in $[Ca^{2+}]_c$.

Aequorin-loaded cells were stimulated by $10^{-5}$ M phenylephrine ( A ), 5 x $10^{-10}$ M vasopressin ( B ) and $10^{-9}$ M angiotensin II (C) in medium containing 0.5 mM ( left ) or 1 $\mu$M ( right ) calcium. Each agent was added as indicated by the arrow.

to both vasopressin and angiotensin II were similar but distinct from that by phenylephrine (Fig. 1B, C). Thus, in response to either vasopressin or angiotensin II there was only a single peak of aequorin luminescence. The duration of the peak was about 30-40 sec. The peak has same rapid rise as that with phenylephrine, but the later phase of the descent of the peak was slower than that by phenylephrine.

Since the difference in the pattern of aequorin luminescence in response to phenylephrine and to either vasopressin or angiotensin II may be due to differences in calcium influx by these agents, we

examined the aequorin luminescence in response to these agonists in the hepatocytes incubated in the medium containinig 1 $\mu$M calcium. In this condition, calcium influx is expected to be negligible (8). In the medium containing 1 $\mu$M calcium, resting $[Ca^{2+}]_c$ was 96 ±12 nM (mean ± S.E., n=8). Figure 2 compares the aequorin luminescence of the hepatocytes in response to these three agonists in the presence of 0.5 mM and 1 $\mu$M $Ca^{2+}$ in the incubation medium.

$\mu$M calcium, the changes in the luminescence in response to phenylephrine were almost identical to those by vasopressin and angiotensin II. Moreover, the heights of the peaks by these agonists are lower and the peaks returned to the baseline faster in the presence of 1 $\mu$M calcium than in the presence of 0.5 mM calcium.

## Discussion

In the present study, the difference in calcium-mobilizing activities between phenylephrine and either vasopressin or angiotensin II is demonstrated by the use of aequorin. In the presence of 0.5 mM extracellular calcium, changes in $[Ca^{2+}]_c$ induced by phenylephrine and either vasopressin or angiotension II are qualitatively different. The phenylephrine-induced elevation of $[Ca^{2+}]_c$ is biphasic whereas those induced by vasopressin and angiotensin II are monophasic. The first peak in response to phenylephrine is steep and it resembles the single peak seen in resone to vasopressin or angiotensin II. However, the duration of the single peak induced by vasopressin or angiotensin II was longer (about 30-40 sec) than the first peak induced by phenylephrine (about 20 sec). In contrast, all three agents induce a qualitatively similar, transient and monophasic elevation of $[Ca^{2+}]_c$ in the presence of 1 $\mu$M extracellular calcium. Considering that these agents do not augument calcium influx under this condition (8), these results suggest that these three agents have comparable effects on calcium release from the intracellular pool(s). This notion is in accordance with recent observations that three agonists increase inositol

1,4,5-trisphosphate in the hepatocytes (4-7). Therefore, the differences in the effects of these three agonists on $[Ca^{2+}]_c$ in the medium containing 0.5 mM may be due to the differences in their effects on calcium influx. Mauger et al. (8) have shown that phenylephrine, vasopressin and angiotensin II stimulate calcium influx to different extents and that the effects of vasopressin and angiotensin II are greater than that by phenylephrine, observations consistent with the present study.

It should be noted that aequorin luminescence was obtained from numerous number of cells in the present study. A recent report examining aequorin luminescence in a single hepatocyte has shown that phenylephrine causes a large burst followed by multiple spikes of $[Ca^{2+}]_c$ (13). It is possible that we may have missed such multiple spikes since the luminescence signals from multiple cells are not precisely synchronized. In any case, the changes in $[Ca^{2+}]_c$ recorded by aequorin luminescence are quite different from those recorded by quin-2. When $[Ca^{2+}]_c$ is measured with aquorin, calcium mobilizing agents induce a prompt and transient increase in $[Ca^{2+}]_c$ while quin-2 fluorescence increases slowly and remains sustained. Recent study in our laboratory has shown that the calcium chelating activity of quin-2 may significantly modify the apparent calcium signal (10). Thus, the failure to detect differences in the actions of three vasoactive agonists on $[Ca^{2+}]_c$ using quin-2 may be due to its calcium chelating activity.

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