Some Agents Which Potentiate the Inotropic Response of the Papillary Muscle to Epinephrine

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The current study was suggested by the observation that various substances are capable of potentiating the inotropic response of the papillary muscle to epinephrine. The object of the study has been to obtain information about the nature of the action by which such substances as cysteine, cystine, tyrosine, ascorbic acid, and plasma are capable of producing this potentiation. Four possible sites of action are considered where such substances might act to alter the response of the papillary muscle to epinephrine. These are: a) the tissue enzymes involved in the destruction of epinephrine; b) the autoxidation of epinephrine; c) the sensitivity of the muscle to epinephrine; d) the general responsiveness of the muscle.

Several different tissue enzymes which destroy epinephrine have been considered in the literature. Blaschko et al. (1) demonstrated that tissues contain a cyanide-insensitive enzyme, amine oxidase, which inactivates epinephrine. Clark and Raventos (2) concluded that the isolated frog auricle will oxidize epinephrine by means of a tissue catalyst. However, these authors did not study the auricle in a bath, but instead, moistened it by topical application with the epinephrine solution. Green and Richter (3) have shown that the cytochrome-indophenol-oxidase system present in all tissues will catalyze the oxidation of epinephrine to adrenochrome. This cytochrome-oxidase system is found in especially high concentrations in cardiac muscle. Inactivation of these enzyme systems should potentiate the action of epinephrine.

The fact that numerous agents inhibit the autoxidation of epinephrine by molecular oxygen in vitro is well known. Such inhibition should also potentiate the epinephrine response. Glutathione (4), cysteine, ascorbic acid (5), plasma (6), and other amino acids (7) inhibit the autoxidation of epinephrine in vitro.

Another possibility considered as a mechanism of action of the potentiators deals with the sensitivity of the muscle to epinephrine. For example, it is conceivable that these substances increase the rate of penetration of epinephrine into the cell or increase the responsiveness of the epinephrine receptors to a given concentration of epinephrine.

Finally it seemed necessary to evaluate the possibility that these substances act by enhancing the general responsiveness of the muscle. Thus they might increase the mechanical response of the contractile element to any type of stimulus.

Observations in the current study emphasize the importance of the role of autoxidation of epinephrine on the magnitude of the response of the papillary muscle when the study is carried out in a bath of Tyrode’s solution. Evidence was obtained which suggests that the potentiation observed was the result of the binding of trace amounts of heavy metals which otherwise catalyze this autoxidation.

METHODS

The data presented was based on experiments using the papillary muscles from 2 dogs and 16 cats. No difference was observed in the response of the muscles of these two animals. Isotonic contractions of the isolated muscle were recorded with a sensitive muscle lever on smoked paper. The muscle was suspended in a 100-cc bath of Tyrode’s solution kept at 38°C. The solution contained 2.1 gm NaHCO₃/l and was bubbled with a mixture of 5% CO₂ in oxygen to maintain a pH between 7.2 and 7.4. A square wave pulse generator was used to stimulate the muscle at a rate of 25-30/min. The average pulse required for stimulation had a duration of 1 msec and a magnitude of 3 v. Two platinum stimulating electrodes were used. One served as the anchor for the lower end of the muscle and the other was placed nearby the muscle in the solution.
The bath could be flushed by siphoning the used solution and adding fresh oxygenated solution from a reservoir which was kept at the same temperature. The muscle was exposed to air for short periods during this procedure. Exposure to air often caused a transient positive inotropic effect (figs 2, 3 and 5) which has been observed by other investigators (8). The different materials studied were added directly to the bath.

**RESULTS**

**Response to Epinephrine.** Krop (9) has shown that epinephrine will produce a positive inotropic response in the isolated papillary muscle of the cat. In our experiments, a small dose of epinephrine (1-5 μg/100 cc) elicited a positive inotropic response which lasted from 3-5 minutes. These responses could be reproduced at 10-minute intervals. It seems probable that there was total destruction of the epinephrine in the bath within the 10-minute interval since there was no evidence of a cumulative effect when a prolonged series of responses was studied. Figure 1 is presented to demonstrate that the response to a given dose of epinephrine remained uniform for over seven hours.

Larger doses of epinephrine produced proportionately greater increases in the strength of contraction and occasionally initiated a spontaneous rhythmicity in the papillary muscle.

**Demonstration of Potentiation.** In order to demonstrate the potentiation of the positive inotropic effect of epinephrine, the potentiating substance was added to the bath 2 minutes before the epinephrine. This 2-minute interval was an arbitrary selection, since the amount of potentiation for a given dose of potentiator was not altered if the substance was added a few minutes before the epinephrine or simultaneously with it. The degree of potentiation could be evaluated by comparing both the height and the duration of the positive inotropic response with these characteristics of the previous control response to epinephrine.

Cysteine was selected for a study of the effect of varying doses of the potentiator on the degree of potentiation (fig. 2). It was found that the height of the potentiated response was proportional to the amount of cysteine in the bath up to a certain concentration. Higher concentrations of cysteine caused no further increase in the height of the response but did increase its duration.

This observation that the potentiator had a greater effect on the duration than on the magnitude of the response was confirmed by the following technique. Cysteine was added early in the declining phase of a positive inotropic response to epinephrine. The response was greatly prolonged, but there was no secondary increase in height of contraction.

These characteristics of the potentiation are in accord with an interpretation that they act by preventing the destruction of epinephrine rather than by making the tissue more sensitive to it. Furthermore, it should be emphasized that the potentiators themselves do not increase the responsiveness of the tissue since even in amounts 20 times as great as a minimal potentiating dose they alone have no positive inotropic effect (figs. 2 and 3).

**Relative Effectiveness of Different Potentiating Agents.** The relative ability of minute amounts of the various materials to potentiate the epinephrine response was established by determining the minimal dose of each required to produce the potentiation. It was found that 3 μg of cysteine (fig. 3), 3 μg of cystine, 100 μg of tyrosine, or 176 μg of ascorbic acid would give minimal potentiations. Plasma (human, dog, cat), in concentrations of 0.005 cc in the 100-cc bath, also gave a minimal
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potentiation (fig. 4). A maximal increase in height of the positive inotropic response was obtained when as little as 0.2 cc is added to the 100-cc bath.

It is interesting to note that it requires no more cystine than cysteine, its reduced form, to potentiate the epinephrine response. Bacq (10) has suggested that the autoxidation of epinephrine might be retarded by these substances because they are oxidized in preference to epinephrine. On this basis cysteine and ascorbic acid should be more effective protectors of epinephrine than cystine since they are much more easily oxidized than cystine. However, in view of the observations in the current study that cystine and cysteine are equivalent potentiators and that both are much more effective than ascorbic acid, it is unlikely that their protective effect in these minute amounts depends on preferential oxidations.

Effect of Potentiating Agents on the Positive Inotropic Response Elicited by Barium Chloride. Barium chloride or barium sulfide (250–1000 μg/100 cc) produced a positive inotropic response (fig. 5). This observation is not in accord with that of Krop (9) who found that BaCl₂ in even higher concentrations (1:100,000–1:10,000) had no effect on the papillary muscle. In the current study the presence of cysteine in the bath did not alter the response of the muscle to barium. The fact that cysteine failed to potentiate the positive inotropic response of the papillary muscle to barium, suggests that this amino acid does not indiscriminately increase a positive inotropic effect.

Amine-Oxidase Inhibitors. That an amine-oxidase system in the cardiac tissue might be operating to destroy epinephrine is a possibility. Inhibition of this enzyme might potentiate an epinephrine response. Benadryl and indolacetic acid have been shown to inhibit amine-oxidase both in vivo and in vitro (11).

In the current study these two inhibitors failed to alter the response of the papillary muscle to epinephrine. Furthermore, they failed to alter the potentiating effects of cysteine on this response. This evidence would indicate that the amine-oxidase system did not affect the epinephrine response in this experiment. Ephedrine is another well known amine-oxidase inhibitor (12). Krop (9) in his studies on the papillary muscle found that ephedrine did not potentiate the epinephrine response. This observation is in accord with the view that the amine-oxidase system is of no significance in the type of epinephrine potentiation currently studied. However, it is well to consider Krop's
might be due to this mechanism. Again, the large excess of epinephrine over the available tissue enzymes under the conditions of the current experiment, should render any such enzymatic action insignificant compared to the rate of destruction of epinephrine by autoxidation.

**Autoxidation of Epinephrine.** The following study was performed to evaluate the relative importance of autoxidation and tissue enzymatic action under the conditions of the current experiments (fig. 6). A dose of epinephrine which was previously demonstrated to elicit a response was incubated away from the tissue in 50 cc of Tyrode's solution at 38°C. The tissue bath was then half emptied, with the muscle remaining immersed in the solution. Next, the 50 cc of incubated solution was added to the tissue bath. By varying the duration of incubation of the epinephrine prior to its addition to the tissue bath, the time required for the destruction of the biological activity of the epinephrine in the absence of tissue enzymes could be determined. It was found that no activity could be demonstrated after 2½ minutes of oxygenation at 38°C. However, when the potentiating substances were also present in the incubated solution, the activity of the epinephrine persisted after 2½ minutes. Results of such studies were comparable to those seen when the potentiation was demonstrated by adding the potentiators and the epinephrine directly to the bath containing the papillary muscle. It would appear that under the conditions of this experiment, the destruction of epinephrine by tissue enzymes is not significant. Instead, the destruction of epinephrine must be accomplished by autoxidation and it is this process that is delayed by the potentiators.

**The Catalytic Action of Copper.** In vitro, epinephrine is rapidly oxidized by molecular oxygen, the oxidation being accelerated by traces of copper, iron, mercury, and other heavy metals, and also by an alkaline pH (14, 15). The buffering effect of the Tyrode's solution used in the current study would prevent any change of pH produced by the small amounts of the potentiating substances which were studied.

Verly (5) has stated that he considers that the phenomenon of inhibition of epinephrine oxidation by the amino acids is due to the formation of copper salts of the amino acids which have a very weak ionization constant. Thus the amino acids take up the cupric ions which would otherwise catalyze the autoxidation of epinephrine. He has shown that the addition of glycine will dissolve the precipitate formed when CuSO4 is added to a phosphate buffer solution of pH 7.5. This reaction is seen only at alkaline pH. Epinephrine can also bind copper at alkaline pH so that there must be a competition between epinephrine and the amino acids for copper. However, the amino acids effectively protect epinephrine against oxidation so that it seems likely that the glycine salt of copper has an ionization constant which is below that of the epinephrine salt of copper.

The role of the cupric ions in the current study was evaluated by the following observations. Small amounts of CuCl2 (27 μg, or 1/10,000 mm) eliminated a standard response of the papillary muscle to epinephrine (fig. 7).
The standard response could be recovered by flushing away the added CuCl₂. It also was found that by adding cysteine (36 μ or 7/10,000 mM) the blocking effect of CuCl₂ could be abolished. In the experiments recorded in Figure 7 a larger amount of cysteine was used to eliminate the effect of copper. In the absence of added copper this dose of cysteine would have caused a marked potentiation of the epinephrine response (Fig. 2). However, in the presence of copper this dose of cysteine did little more than reestablish the standard response to epinephrine.

It would appear that the blocking effect of CuCl₂ was due to the very rapid oxidation of the epinephrine, the cupric ions acting to catalyze the reaction. That this blocking effect of copper could be removed by an adequate amount of cysteine is good evidence that cysteine combines with copper and thus retards the oxidation of the epinephrine. The simple removal of the copper from the bath by flushing had essentially the same effect.

A similar relationship between copper and a copper binding substance has been observed by Dantas and Chenoweth (16) in their study of the response of the nictitating membrane to neurogenic stimulation. They have shown that copper chloride abolished the lowered threshold produced by hesperidin methyl chalcone. It was suggested that the effect of the adrenergic substance involved in the contraction of the intact nictitating membrane is decreased by the presence of copper and that hesperidin methyl chalcone produces its potentiating effect by binding the copper.

FIG. 6. Top: epinephrine incubated in Tyrode's solution for 2.5 min. loses its biological activity. This loss is prevented by the presence of cysteine.

FIG. 7. Bottom: response of the papillary muscle to epinephrine can be eliminated by the addition of CuCl₂ to the Tyrode's solution. Subsequent addition of cysteine prevents this action of copper.

demonstrated that the intravenous injection of ascorbic acid failed to alter the response of the blood pressure on the nictitating membrane to epinephrine. Since there is a great surplus of potentiating substances (inhibitors of autoxidation) in the blood, it is unlikely that any reasonable variation in their concentrations would alter the rate of autoxidation of epinephrine in the blood stream. The presence of these substances in the blood allows circulating epinephrine to reach its sight of action without undergoing autoxidation.

SUMMARY AND CONCLUSIONS

Cysteine, cystine, tyrosine, ascorbic acid, and plasma potentiate the positive inotropic response of the papillary muscle to epinephrine. The positive inotropic response elicited by BaCl₂ is not altered by cysteine, thus sug-
gesting that the general responsiveness of the muscle is not affected by this substance. The amine-oxidase inhibitors, benadryl and indolacetic acid did not potentiate the epinephrine response.

The rate of destruction of the biological activity of epinephrine in the absence of the cardiac tissue is retarded by cysteine and ascorbic acid. The presence of the cardiac tissue appears to have no significant role in the destruction of epinephrine under the conditions of these experiments. The potentiating agents studied will effectively neutralize the blocking action of copper on epinephrine. The probable mode of action of these potentiating agents is the removal of trace amounts of heavy metals which catalyze the autoxidation of epinephrine.

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REFERENCES