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Ethacrynic Acid Induced Inotropism[1] (38958)

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(Introduced by Y. Wang)

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Ethacrynic acid (ECA) is frequently administered in the presence of acute pulmonary edema. The major mechanisms of improvement in such patients are thought to be an acute reduction of blood volume stemming from the diuresis induced by the drug and drug-induced vasodilation (1, 2). Until recently there has been little direct exploration of the possibility that ECA might also have a direct cardiac effect (3–6). Our own previous work with *N*-ethyl maleimide (NEM) (7) and *P*-chloromercuribenzene sulfonic acid (PCMBS) (8), two classical sulfhydryl group inhibitors demonstrated they had positive inotropic properties. Because of the striking hemodynamic response frequently following the administration of ECA and because the compound is known to act by inhibiting sulfhydryl groups (9), ECA was evaluated for possible positive inotropic properties.

Methods. Isolated guinea pig left atria were placed in a tissue bath and isometric tension was measured with a strain gauge transducer (Statham, UC3) and recorded with an oscillographic recording system (Sanborn 351). Tyrode's solution containing 4 m*M* K and either 1.8 m*M* or 0.9 m*M* Ca was utilized as the bath medium and was bubbled with a 95% O_2 and 5% CO_2 mixture which maintained the pH at approximately 7.3. The bath temperature was maintained at 37°. The muscles were paced at 160 beats/min at a voltage 10% above the threshold, utilizing large platinum field electrodes. The preload (diastolic tension) was set at approximately 300 mg in each experiment.

Drug solutions were made up daily. When reserpine (R) was administered to deplete the cardiac catecholamines, a dose of 5 mg/kg was injected ip, 24–36 hours prior to removal of the atria (10). As indicated, isometric contractile tension was measured, and except where indicated, the results are presented as percent of control (zero time) active tension ± the standard error of the means (SEM).

Results. Average control active tension in the series of experiments carried out in the 1.8 m*M* Ca bath was 509 ± 109 mg. Figure 1 demonstrates the abrupt but transient increase in contractile tension in this group of atria following the addition of ECA (2×10^{-4} *M* bath concentration). Within 60 min following administration of the drug, contractile tension dropped below that of the untreated control group. Peak tension response was 149 ± 11% of control ($P < .005$ Student's *t* test) and occurred at 3.1 ± 0 min following administration of the drug. Diastolic tension rose to 707 ± 117 mg by 120 min post ECA treatment from a control level of 296 ± 14 mg ($P < .005$). Also shown in Fig. 1 are a group of untreated atria observed for 90 min under comparable circumstances. There was a gradual fall in active tension in this group but no change in diastolic tension.

In the 0.9 m*M* calcium bath, the control active tension was 121 ± 18 mg. As seen in Fig. 2, there was an enhanced drug response under these conditions with a rapid rise to a peak response of 415 ± 109% of control tension ($P < .01$) at 3 ± 0 min. Though the positive inotropic effects of ECA are of longer duration, the tension ultimately fell below that of the control group. Diastolic tension rose from 300 ± 8 mg to 668 ± 72 mg at 120 min ($P < .001$) after introduction of the drug. An untreated group of atria, also shown in Fig. 2 showed a gradual

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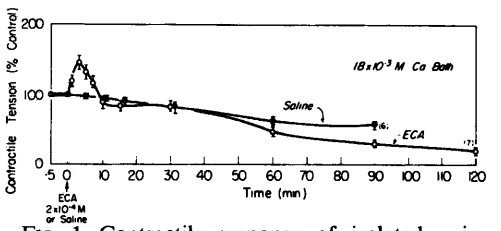

FIG. 1. Contractile response of isolated guinea pig left atria treated with (2×10^{-4} *M*) ECA and that of an untreated group of atria in a 1.8 m*M* Ca bath. I = SEM.

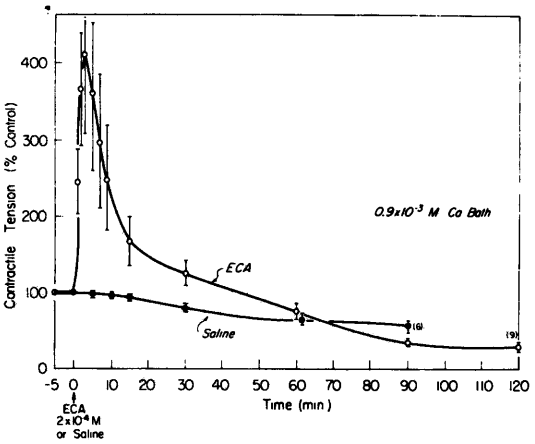

FIG. 2. The same experiment as shown in Fig. 1 carried out in a 0.9 m*M* Ca bath.

decrease in active tension and no change in diastolic tension.

The effects upon ECA-induced inotropism of propranolol (P) pretreatment of atria (1 μg/ml bath concentration) are seen in Figs. 3 and 4. In Fig. 3, the experiments are carried out in a 1.8 m*M* calcium bath and the peak response of the atria to ECA is virtually unchanged. The peak tension rise was 149 ± 11% of control in the ECA group alone and 143 ± 14% of control in the ECA plus P group and the difference was not significant. The final diastolic tension of the two groups was also comparable (707 ± 117 mg in the ECA group and 694 ± 218 mg in the ECA plus P group at 120 min). Figure 4 shows experiments carried out in a 0.9 m*M* calcium bath with the group of reserpine-treated atria also included. In contrast to the data shown in Fig. 3, P-induced β-blockade and reserpine-induced catecholamine depletion caused marked comparable reductions in the peak

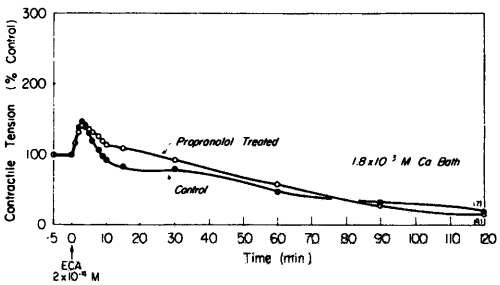

FIG. 3. The contractile response of two groups of atria treated with ECA (2×10^{-4} *M*) with one group exposed to propranolol (1 μg/ml bath concentration) in a 1.8 m*M* Ca bath.

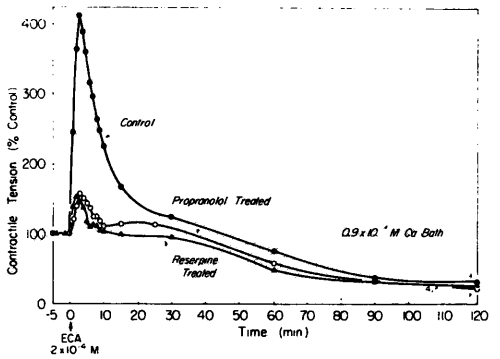

FIG. 4. The same experiment as shown in Fig. 3 carried out in a 0.9 m*M* Ca bath. There is an additional group of atria shown obtained from animals pretreated with 5 mg/kg reserpine, 24–36 hr before use.

inotropic response (415 ± 109% of control in the ECA group, 163 ± 16% of control in the ECA plus P group, and 157 ± 15% in the ECA plus R group). The difference between the ECA control group and ECA plus P group was significant ($P < .05$), but the difference between the ECA control group and the ECA plus R group was not ($P < .10$ but $> .05$). The 120 min diastolic tensions of all groups were comparably elevated (ECA group 668 ± 72 mg; ECA plus P group 652 ± 96 mg; and ECA plus R group, 713 ± 66 mg). The times to peak inotropic response were not modified by any of the drug treatments.

Discussion. It is clear from the data presented that ethacrynic acid does have a positive inotropic effect upon cardiac muscle and subsequent to our preliminary report (4), these observations have been confirmed (5, 6).

The enhancement of the contractile response in a "low" calcium environment is not surprising being seen with both cardiac glycosides (11) and catecholamines (12) and presumably relates to the calcium dependence of these effects.

The mechanism of the ECA-induced inotropic response is not entirely clear. It is apparent that there is a catechol release related component (best demonstrated in the "low" rather than normal Ca bath), and this observation has been confirmed (6). This property is shared in common with NEM (7) which has a distinctly biphasic response, the first component of which is catecholamine dependent. NEM causes release of catecholamines from adrenal granules (13) and it is likely that NEM and ECA do the same at sympathetic nerve endings.

The noncatecholamine-dependent component of the inotropic response is of more theoretic interest. Biochemical investigations would support several potential explanations. ECA is known to be *in vitro* and *in vivo* inhibitor of the Na–K-dependent-ATPase in several tissues (14). Inhibition of this enzyme is thought by some to underlie the inotropic response of cardiac muscle to cardiac glycosides (15), and it is possible that ECA acts in this fashion as well. It is also possible that ECA acts by increasing calcium influx independent of the ATPase inhibition, perhaps by altering cell membrane permeability to calcium or other cations. As ECA is a small relatively permeable molecule, it may also act intracellularly to alter stored calcium release, diminish calcium uptake of the sarcoplasmic reticulum or even possibly act upon the contractile myofilaments directly. There is also some evidence that ECA is a phosphodiesterase inhibitor (3) and a resulting rise in intracellular cyclic AMP could also cause an increase in contractility. Because, however, nonpermeable PCMBS is positively inotropic (8), a cell membrane event such as Na–K-dependent ATPase inhibition or a directly induced increase in calcium permeability would seem the best explanation for the noncatechol dependent inotropic effects of ECA and different intracellular mechanisms or membrane events likely to account for the accelerated failure of contraction in the presence of ECA.

The failure of active contraction induced by ECA may be explained by the ability of this compound to inhibit enzyme sequences underlying both aerobic and anaerobic metabolism (16, 17) or by cell membrane destruction (18) with subsequent loss of function. Inhibition of metabolism alone, however, will not account for the increased diastolic tension (contracture) observed since relatively complete inhibition of oxidative metabolism with cyanide does not cause this response even with all contractile tension abolished (19). Late contracture is also seen with other sulfhydryl compounds which are capable of penetrating the cell membrane, notably NEM (7) and iodoacetic acid (19) and with cardiac glycosides in toxic doses (20). PCMBS, a nonpenetrating sulfhydryl inhibitor which was capable of causing a prolonged inotropic response in a comparable muscle preparation, did not cause contracture even after the inotropic response had faded over a period of several hours (8) suggesting that an intracellular action of sulfhydryl compounds is required for this response. The question whether the contracture is caused by actual interference with the contractile apparatus by the sulfhydryl binding reagent (21) or whether it is caused by a drug-induced elevation of cytosol calcium or by other mechanisms remains unanswered.

Although it has been shown that in an isolated cardiac preparation that ECA is positively inotropic, this effect is probably not seen in the clinical situation. A brief calculation would suggest that even following the rapid intravenous injection of the usual dose of ECA and assuming only extracellular distribution, the concentration of ECA available to the myocardium would be well below the level found to be inotropic in this study. Thus, though of pharmacologic interest, it does not seem likely that either the early positive or late negative inotropic effects of ECA described are of clinical significance.

Summary. Ethacrynic acid (ECA), a sulfhydryl group inhibiting diuretic was examined for positive inotropic effects. These were found to be present in isolated

guinea pig left atria studied in 0.9 and 1.8 m*M* Ca bathing solutions and were partially dependent upon adrenergic mechanisms (presumably secondary to norepinephrine release from sympathetic nerve endings) and partly independent of such mechanisms as demonstrated by propranolol induced β-blockade and reserpine-induced catecholamine depletion. The mechanism of the non-β adrenergic inotropism is unclear but may relate to the ability of ECA to inhibit the sarcolemmal Na–K–Mg-dependent ATPase. ECA-induced premature contractile failure occurred in all atria as well as a late increase in diastolic tension, the latter being comparable to that described for toxic doses of cardiac glycosides in similar preparations.

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