Brit. J. Pharmacol. (1962), **18**, 588–594.

EFFECT OF RESERPINE PRETREATMENT ON THE RESPONSE OF ISOLATED PAPILLARY MUSCLE TO EPHEDRINE

BY

V. J. CAIROLI, J. F. REILLY AND J. ROBERTS

From the Department of Pharmacology, Cornell University Medical College, N.Y.C. 21, N.Y., U.S.A.

(*Received January* 30, 1962)

Reserpine pretreatment of cats abolished the action of ephedrine to induce spontaneous beating in papillary muscles obtained from these animals. This spontaneous beating was viewed as resulting solely from catechol amine release. The positive inotropic action of ephedrine was diminished but not abolished by reserpine pretreatment. Reserpine reduced the heart rate *in vivo*, but it did not affect the peak contraction height or the rate of failure of the isolated papillary muscle. The contractile response to adrenaline (0.1 μg/ml.) was not affected by reserpine pretreatment. However, in this concentration adrenaline induced spontaneous beating only after reserpine.

There is considerable evidence that reserpine causes catechol amine depletion of cardiac muscle (Bertler, Carlsson & Rosengren, 1956; Paasonen & Krayer, 1958; and Lee & Shideman, 1959). Burn & Rand (1958a) and Innes & Krayer (1958) reported that the sinus rate was reduced as a consequence of this depletion. In addition, Lee & Shideman (1959) showed that myocardial contractility was diminished after reserpine pretreatment.

Roberts & Modell (1961) demonstrated in dogs with complete heart block that catechol amines were of greater importance in ventricular than in atrial rhythmicity; furthermore, Roberts & Stadter (1960) showed that catechol amine depletion by reserpine sharply reduced the ability of the ventricle to escape from sinus control during vagal stimulation. The importance of amines in ventricular rhythmicity suggested that agents affecting ventricular activity exert their effects, at least in part, through catechol amine release. In this regard, a possible relationship was demonstrated between the effect of quinidine and ouabain on ventricular rhythmicity and catechol amine activity (Stadter, Olichney, Roberts & Modell, 1960; Cairoli, Reilly & Roberts, 1961).

To explore further this relationship between catechol amines and the effects of cardioactive agents on the ventricle, the actions of ephedrine were investigated in papillary muscles obtained from cats pretreated with reserpine. There is evidence indicating that the ephedrine acceleration of sinus rate (Bejrablaya, Burn & Walker,

1958; Innes & Krayer, 1958) is related to catechol amine release. The results of our experiments indicate that the effect of ephedrine on the contractility and rhythmicity of the ventricle is also largely due to catechol amine release.

METHODS

Cats were rendered unconscious with electroshock by applying 140 V across the temporal regions of the skull. The current flow between the two electrodes was 110 mA. Papillary muscles were removed from the right ventricle according to the technique of Cattell & Gold (1938). The muscles were placed in a holder between chlorided silver electrodes, and isometric contractions were recorded in a manner similar to that described by Garb & Chenoweth (1953). The muscle was immersed in a glucose-free solution described by Krebs & Henseleit (1932) through which 95% oxygen and 5% carbon dioxide was bubbled; the bath was maintained at 37° C and a *p*H of 7.4 with a bicarbonate buffer. Maximal stimuli of square-wave pulses 0.5 to 1 msec duration at 4 to 10 V and at a frequency of 60/min were applied across the base of the muscle. The amplitude of contraction was measured on a mm scale by observing the deflection of a light beam reflected from a mirror attached to an isometric lever; the tension was adjusted to produce optimal amplitude of contraction. Each mm of deflection represents 7 mg of tension; this relationship remained constant up to a deflection of 300 mm. The muscles were allowed to fail until a steady level was attained. On the average (75 muscles) this occurred in 1 to 2 hr and a contraction height of 46% (s.e. $\pm$1.6) of the initial amplitude. At this time, solutions of drugs were added in volumes which did not exceed 1/100th of the total bath volume (100 to 120 ml.). The *p*H of the bath was not altered by the addition of the drugs. Concentrations of ephedrine and L-adrenaline are expressed in terms of their free base. Reserpine (Serpasil, Ciba) 10 mg/kg was injected intraperitoneally 24 to 26 hr before the experiment. At the conclusion of most experiments the wet weight of the papillary muscle was determined. The average wet weight for 37 muscles was 37 mg (s.e. $\pm$2.8). Before electroshock was applied, the heart rate was determined by recording the electrocardiogram using a Cambridge simpli-scribe electrocardiograph. The standard error of the mean is indicated after all averages.

RESULTS

Effects of reserpine. Reserpine pretreatment, as expected, greatly diminished the heart rate of the intact animal. In the 16 untreated animals the average heart rate was 177 $\pm$ 6.7 beats/min, while in 24 reserpine-treated animals it was 80 $\pm$ 4.7 beats/min. Nevertheless, reserpine pretreatment did not affect the contractility of the papillary muscles excised from these animals. Thus, the average peak amplitude of contraction developed by 53 untreated muscles was 166 mm $\pm$ 10.8 compared with 163 mm $\pm$ 8.6 in 46 reserpine-treated muscles. Nor, as may be seen in Fig. 1, did reserpine influence the initial amplitude of contraction (153 mm $\pm$ 11.9 for untreated muscles and 158 mm $\pm$ 10.3 for treated muscles) or the rate of failure.

Effects of ephedrine. Ephedrine in concentrations of 0.4 to 40 μg/ml. (Table 1) induced spontaneous beating in 13 of 31 papillary muscles. After reserpine, however, this effect of ephedrine was completely absent (0/32). Ephedrine also produced an increase in the force of contraction of the untreated papillary muscles (Table 1). This effect of ephedrine on the contractility of papillary muscle was noted by Krop (1944). It may be seen from this table that a concentration of 0.04 μg/ml. was necessary to elicit a minimal response, while the maximum response was approached beginning with a concentration of 0.4 μg/ml. Since some muscles developed spontaneous beating in the range 0.4 to 40 μg/ml., it was possible that the full

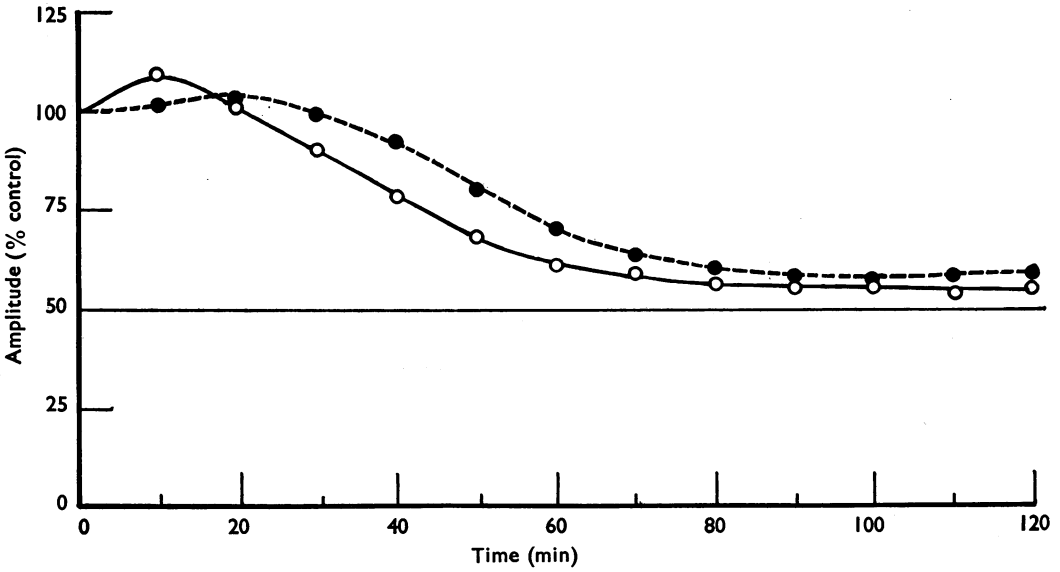

Fig. 1. The effect of reserpine on the rate of failure of the cat papillary muscle. From 0 to 80 min, each point on the curve of the untreated muscles (○——○) represents the average of 53 experiments; while after 80 min, each point represents the average of 53 to 19 experiments. From 0 to 80 min, each point on the curve of the reserpine treated muscles (●- - -●) represents the average of 45 experiments; while after 80 min, each point represents the average of 45 to 27 experiments. There is no significant difference between the points of the untreated and reserpine-treated series (Student's "t" test, $P > 0.10$). Reserpine was administered intraperitoneally 10 mg/kg 24 to 26 hr prior to the experiment. Average initial amplitude of contraction for untreated muscles was 153 mm±11.9 and for reserpine-treated muscles was 158 mm±10.3. The amplitude is expressed as a % of these initial values.

development of the ephedrine action on contractility was not observed. However, it was found that the omission of these muscles did not significantly change the mean response at any of these concentrations; therefore, these muscles were included in the averages (Table 1).

TABLE 1

EFFECT OF RESERPINE PRETREATMENT ON THE ACTION OF EPHEDRINE IN THE CAT PAPILLARY MUSCLE

Spontaneous beating occurred in the presence and in the absence of evoked contractions. Periods of spontaneous beating of less than 1 min are not included

	Untreated			Reserpine-treated		
Ephedrine (μg/ml.)	No. of muscles	Average increase in amplitude (mm±s.e.)	Incidence of spontaneous beating	No. of muscles	Average increase in amplitude (mm±s.e.)	Incidence of spontaneous beating
0·04	6	7±0·50	0/6	—		
0·08	6	23±4·3	0/6	—		
0·40	10	41±8·9	6/10	10	3±1·4	0/10
4·0	11	65±8·8	4/11	11	17±3·1	0/11
40·0	10	73±15·4	3/10	11	23±3·8	0/11

After reserpine, the threshold concentration of ephedrine was increased and the maximum contractile response was lowered (Fig. 2). A comparison of the responses evoked by the same concentrations of ephedrine in the treated and untreated series, employing analysis of variance, showed them to be significantly different ($P<0.001$).

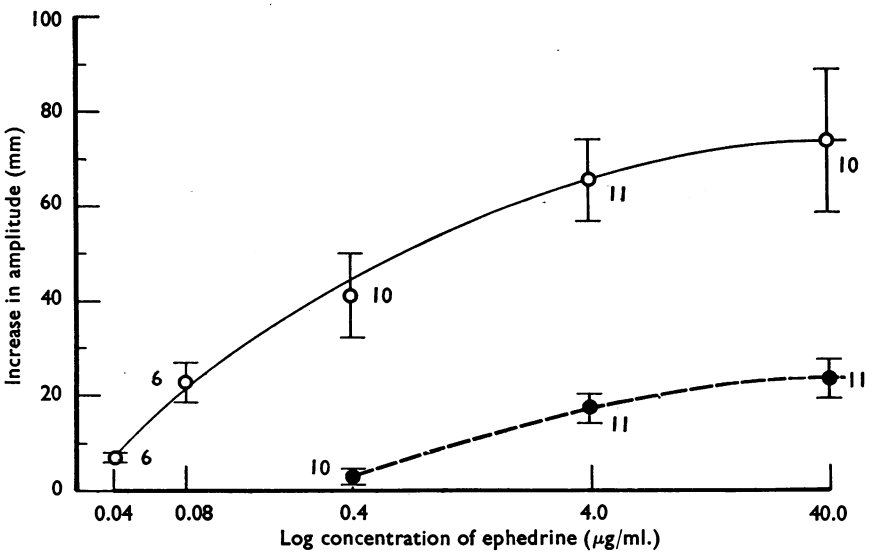

Fig. 2. Concentration-response curves of ephedrine in untreated (○ —— ○) and reserpine-treated (● - - - ●) muscles. Each point represents the average of the observed responses with the standard error of the mean shown by the vertical line above and below the point. The figures indicate the number of observations. The ordinate represents the increase in amplitude of contraction produced by ephedrine in papillary muscles which had failed to a steady level.

Since reserpine is known to deplete catechol amine stores (Bertler *et al.*, 1956; Muscholl & Vogt, 1958; and Lee & Shideman, 1959), the above results would accord with the view that the response to ephedrine is diminished by a reduction in these stores. The increase in the threshold concentration after reserpine may indicate that either larger concentrations of ephedrine are required to release effective amounts of catechol amine from the reduced stores and/or that larger concentrations are required to elicit a direct action.

Effects of adrenaline. To explore the possibility of a direct depression by reserpine of the contractile mechanism, adrenaline (0.1 μg/ml.) was administered to both untreated and reserpine-treated muscles. The results are shown in Table 2. It is apparent that the positive inotropic response to adrenaline was in no way affected. This observation supports other reports in which the effects of certain sympathomimetic amines on other tissues treated with reserpine were either diminished or entirely abolished, but those of noradrenaline or adrenaline were potentiated or not affected (Burn & Rand, 1958b; Fleming & Trendelenburg, 1961). The only suggestion of supersensitivity in the present study was the appearance of adrenaline-induced spontaneous beating after reserpine.

Additional evidence indicating that the contractile mechanism was still responsive after reserpine is shown by the experiments in which adrenaline was administered after the muscles had been exposed to ephedrine and subsequently recovered from the ephedrine effects (Table 2). Despite the relative refractoriness to ephedrine (Table 1), the magnitude of the adrenaline effect was comparable to that seen in the untreated controls.

TABLE 2

EFFECT OF ADRENALINE (0·1 μG/ML.) ON UNTREATED AND RESERPINE-TREATED CAT PAPILLARY MUSCLES BEFORE AND AFTER EPHEDRINE

Ephedrine (μg/ml.)	No. of muscles	Average increase in amplitude (mm±s.e.)	Incidence of spontaneous beating
Untreated			
0	9	79±12·6	0/9
Reserpine-treated			
0	8	76±12·5	3/8
0·40	7	96±19·7	3/7
4·0	8	85±17·2	1/8
40·0	8	56±10·1	2/8

The potentiation of the adrenaline response by ephedrine, reported by other investigators (Schaumann, 1928; Koppanyi & Luckhardt, 1931; Gaddum & Kwiatkowski, 1938), was suggested by the appearance of adrenaline-induced spontaneous beating after ephedrine pretreatment. Thus, there were no adrenaline-induced spontaneous beats before ephedrine in 9 experiments, while after ephedrine treatment they appeared in 13 of 19 experiments (not shown in the tables). The interaction of ephedrine and adrenaline on the contractile response in non-reserpinized muscles could not be accurately evaluated, since the number of papillary muscles which returned to control values after ephedrine treatment was too small for reliable comparison.

DISCUSSION

The most striking finding of this investigation is the failure of ephedrine to induce spontaneous beating after reserpine. Reserpine pretreatment has been shown to deplete cat papillary muscle of catechol amines (Lee & Shideman, 1959). Consequently, the inability of ephedrine to cause spontaneous beating in catechol-amine-depleted tissue clearly implies that the ephedrine-induced spontaneous beating is mediated through catechol amine release. Since adrenaline caused spontaneous beating in papillary muscles which were not responsive to ephedrine, blockade of cardiac adrenergic receptors by reserpine was not a contributing factor.

The positive inotropic action of ephedrine was diminished but not completely abolished in catechol-amine-depleted cardiac muscle. Accordingly, only part of this ephedrine action can be definitely attributed to catechol amine release. The mechanism by which ephedrine exerted an effect on contractility after reserpine is uncertain. This residual effect may be due to release of amines still present after reserpine, to a direct action or to both of these.

The possibility that ephedrine may exert an action on the contractile mechanism not related to activation of catechol amines is suggested by the observation that reserpine pretreatment completely abolished the action of ephedrine to induce spontaneous beating. Thus, if residual catechol amines were responsible for the effect of ephedrine on contractility after reserpine, then it might be expected that they would also provide the means for ephedrine in large doses to induce spontaneous beating. The possibility of a direct action of ephedrine on papillary muscle receives some support from the work of Leusen & Verbeke (1960). They showed that the positive inotropic effect of tyramine was completely abolished by reserpine. However, these workers did not explore the effects of tyramine over a wide concentration range and it is possible that larger doses of tyramine might have still produced a contractile effect in the same way as ephedrine.

Several reports have indicated that after reserpine treatment the catechol amine stores are not completely depleted (Muscholl & Vogt, 1958 ; Lee & Shideman, 1959) ; therefore, it is possible that these residual catechol amines may account in part for the residual effects of ephedrine. Trendelenburg & Fleming (1960) reported that, although the sensitivity of the nictitating membrane to ephedrine was diminished by reserpine pretreatment, nearly maximal responses could be obtained by increasing the doses of ephedrine. Accordingly, these investigators suggested that ephedrine exerted a direct action on the nictitating membrane in addition to that attributable to catechol amine release. In the present study, the maximum contractile response to ephedrine was considerably less after reserpine, suggesting that a direct action was not largely responsible for the residual effect of ephedrine. In any case, the present study supports the earlier suggestion of Burn (1932) that ephedrine action is related to adrenaline release from local stores.

Reserpine did not affect the peak contraction height or the rate of failure of the papillary muscle. Therefore, the results of this investigation are in accord with the studies of Fawaz (1961), which showed that reserpine treatment did not alter the mechanical efficiency of the heart, and differ from those of Lee & Shideman (1959), who reported that reserpine reduced the contractility of papillary muscles.

The reserpine cardiotoxic effects reported by Withrington & Zaimis (1961) did not appear to be an important factor in our experiments, since the initial and peak contraction heights, the rate of failure and the contractile response to adrenaline were not influenced by reserpine pretreatment.

One of us (V. J. C.) is now a Public Health Service Pre-doctoral Fellow (Training Grant 2G-99). One of us (J. R.) is recipient of a grant (H.1165) from the United States Public Health Service, National Heart Institute.

REFERENCES

BEJRABLAYA, D., BURN, J. H. & WALKER, J. M. (1958). The action of sympathomimetic amines on heart rate in relation to the effect of reserpine. *Brit. J. Pharmacol.*, **13**, 461–466.

BERTLER, A., CARLSSON, A. & ROSENGREN, E. (1956). Release by reserpine of catecholamines from rabbit hearts. *Naturwissenschaften*, **43**, 521.

BURN, J. H. (1932). The action of tyramine and ephedrine. *J. Pharmacol. exp. Ther.*, **46**, 75–95.

BURN, J. H. & RAND, M. J. (1958a). Action of nicotine on the heart. *Brit. med. J.*, **i**, 137–139.

BURN, J. H. & RAND, M. J. (1958b). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

CAIROLI, V., REILLY, J. & ROBERTS, J. (1961). The effect of reserpine pretreatment on the positive inotropic action of ephedrine and ouabain. *Fed. Proc.*, **20**, 122.

CATTELL, M. & GOLD, H. (1938). The influence of digitalis glucosides on the force of contraction of mammalian cardiac muscle. *J. Pharmacol. exp. Ther.*, **62**, 116–125.

FAWAZ, G. (1961). The effect of mephentermine on isolated dog hearts, normal and pretreated with reserpine. *Brit. J. Pharmacol.*, **16**, 309–314.

FLEMING, W. W. & TRENDELENBURG, U. (1961). The development of supersensitivity to norepinephrine after pretreatment with reserpine. *J. Pharmacol. exp. Ther.*, **133**, 41–51.

GADDUM, J. H. & KWIATKOWSKI, H. (1938). The action of ephedrine. *J. Physiol. (Lond.)*, **94**, 87–100.

GARB, S. & CHENOWETH, M. B. (1953). The T deflection of isolated mammalian heart muscle electrogram. *Circulation Res.*, **1**, 135–144.

INNES, I. R. & KRAYER, O. (1958). Studies on veratrum alkaloids. XXVII. The negative chronotropic action of veratramine and reserpine on the heart depleted of catecholamines. *J. Pharmacol. exp. Ther.*, **124**, 245–251.

KOPPANYI, T. & LUCKHARDT, A. B. (1931). Studies on the hemodynamic action of subcutaneously injected epinephrine II. Experimental proof of the validity of certain interpretations made on epinephrine action. *Arch. int. Pharmacodyn.*, **40**, 344–356.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkorper. *Hoppe-Seyl. Z.*, **210**, 33–66.

KROP, S. (1944). The influence of heart stimulants on the contraction of isolated mammalian cardiac muscle. *J. Pharmacol. exp. Ther.*, **82**, 48–62.

LEE, W. C. & SHIDEMAN, F. E. (1959). Role of myocardial catecholamines in cardiac contractility. *Science*, **129**, 967.

LEUSEN, I. & VERBEKE, R. (1960). The action of sympathomimetic amines on the myocardium after pretreatment with reserpine. *Arch. int. Pharmacodyn.*, **125**, 246–247.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol. (Lond.)*, **141**, 132–155.

PAASONEN, M. K. & KRAYER, O. (1958). The release of norepinephrine from the mammalian heart by reserpine. *J. Pharmacol. exp. Ther.*, **123**, 153–160.

ROBERTS, J. & MODELL, W. (1961). Pharmacological evidence for the importance of catecholamines in cardiac rhythmicity. *Circulation Res.*, **9**, 171–176.

ROBERTS, J. & STADTER, R. P. (1960). Effect of reserpine on ventricular escape. *Science*, **132**, 1836–1837.

SCHAUMANN, O. (1928). Über den Wirkungsmechanismus der Ephedrins und den Unterschied in der Wirkungsstärke zwischen seinen Isomeren. *Arch. exp. Pharmak.*, **138**, 208–218.

STADTER, R., OLICHNEY, M., ROBERTS, J. & MODELL, W. (1960). Chronotropic responses to quinidine in dogs with complete heart block. *Fed. Proc.*, **19**, 115.

TRENDELENBURG, U. & FLEMING, W. W. (1960). Subsensitivity to certain sympathomimetics after pretreatment with reserpine. *Fed. Proc.*, **19**, 284.

WITHRINGTON, P. & ZAIMIS, E. (1961). The reserpine-treated cat. *Brit. J. Pharmacol.*, **17**, 380–391.