Effect of Reserpine on ^{22}Na and ^{42}K Exchange in the Cat Papillary Muscle[1,2] (35099)

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There are numerous reports which indicate that pretreatment with reserpine reduces the capacity of digitalis to induce arrhythmia (1–4). It has been suggested that this antiarrhythmic effect of reserpine may be due to a depressant action on myocardial excitability similar to that of quinidine (4). Since the antiarrhythmic activity of quinidine is associated with changes in Na and K transmembrane fluxes (5, 6), it was reasoned that if the effect of reserpine to antagonize digitalis-induced ventricular arrhythmias is related, at least in part, to a quinidine-like action, then reserpine pretreatment should cause changes in ion fluxes similar to those produced by quinidine. The present investigation was undertaken to determine the effects of reserpine on Na and K exchange in cardiac muscle.

Methods. All experiments were performed on papillary muscles removed from cats weighing between 1.5 and 2.5 kg. The cat was rendered unconscious with an electric shock of 90–100 V applied for 10–12 sec across the skull between the temporal bones. Papillary muscles were isolated from the right ventricle and placed in a chamber containing Krebs–Ringer solution bubbled with 95% O_2–5% CO_2. Only papillary muscles of less than 1 mm in diameter were used in this study. Krebs–Ringer solution was made up in triple distilled water and consisted of (mmoles/liter): Na^+, 145; K^+, 6; Ca^{2+}, 1.1; Cl^-, 127; Mg^{2+}, 1.2; HCO_3^-, 25; $H_2PO_4^-$, 1.2; SO_4^{2-}, 1.2; and glucose, 5.6 (7). The pH of the solution was kept between 7.4–7.5 and the temperature of the bath was maintained at 37.5° throughout the experiment. The chorda tendinae were attached to a strain gauge [Statham Universal transducer cell (UC2)] and the resting tension was set at 0.5–0.6 g. The muscle was allowed to equilibrate with the Krebs–Ringer solution for a period of 2 hr before it was incubated with the solution containing the radioactive isotope (8). The muscles were stimulated through bipolar platinum electrodes with a stimulus which was as close to threshold as possible. The stimulus was 5–6 V at a pulse duration of 0.5–1 msec delivered at a frequency of 60/min by a Grass stimulator (SD5).

The methods of Holland and Klein and their co-workers were employed to determine ionic exchanges (5, 6, 9, 10). To measure potassium efflux, the fluid bathing the muscle was replaced with Krebs–Ringer solution containing ^{42}K ($^{42}K_2CO_3$, HSA, Nuclear Science and Engineering) in concentrations sufficient to provide 40,000 cpm/ml. All readings were corrected throughout the course of the experiment for radioactive decay of ^{42}K (half-life 12.5 hr). The muscle was incubated with ^{42}K for 6 hr and at the end of this period it was washed every 2 min with fresh nonradioactive Krebs–Ringer solution over a 10-min period. Thereafter, the bath solution was replaced every 10 min, with fresh nonradioactive Krebs–Ringer solution and the radioactivity in the exchanged fluid was determined. This was done by taking a 1-ml aliquot of the exchanged fluid, evaporating it to dryness in a planchet and counting the

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radioactivity in the sample over a 1-min period. Three successive 1-min counts were made and the values used in the calculations represent the average of these counts. Radioactivity in the muscle was determined at the end of the efflux experiments. Before determining radioactivity in the muscles, they were first washed every 2 min over a 10-min period with fresh nonradioactive Krebs–Ringer solution. Radioactivity was measured by placing the muscle in a suitable counting tube containing 3 ml of Krebs–Ringer solution bubbled with 95% O_2–5% CO_2. The counts were made over a 1-min period and the average of two such counts was used in the calculations.

Radioactivity was measured by a 2-in. thallin-activated sodium iodide crystal, Well-scintillation counter system [Nuclear Chicago Analyzer/Scaler (Model No. 8725)].

^{42}K uptake was studied by measuring muscle counts 1, 3, and 6 hr after the muscle was first exposed to ^{42}K solution. The method for determining muscle counts was the same as described above.

^{22}Na uptake (^{22}NaI, HSA, Nuclear Science and Engineering, half-life 2.5 years) was determined by incubating the muscle with sufficient amounts of ^{22}Na so that 1-ml of the bath solution would provide counts of approximately 40,000/min. After every 20 min of incubation, a 1-ml aliquot was withdrawn from the bath and the radioactivity of this sample was counted over a 1-min period. Three such counts were made on each sample and the average of these counts was used in the calculations. After counting, the sample was returned to the bath. The muscle counts were determined at the end of the Na uptake studies and were performed in the same manner as previously described in the K efflux studies.

^{22}Na efflux was determined using essentially the same methods as those employed in the measurement of ^{42}K efflux except the bath fluid was changed every 15 min and the counting was performed in a glass counting tube. The muscle was incubated for 3 hr with ^{22}Na since the uptake of radioactive sodium reaches a maximum at this time (see Results). Muscle counts were determined at the end of the efflux studies using the same method that was employed in the ^{42}K experiments.

Total potassium and sodium content of the muscle were measured by flame photometry (Baird Atomic flame photometer) using a method similar to that of Hercus *et al.* (11). The values are expressed in terms of meq/liter of tissue H_2O.

Total water content of the muscle was determined prior to immersion in Krebs–Ringer solution and at the end of the efflux or influx studies by first gently blotting the muscle with filter paper, taking its weight and then drying it overnight in an oven set at 120°. The muscle was allowed to cool in a desiccator and was subsequently weighed to determine its dry weight. The percentage total water of the muscle was calculated by the following formula:

$$\% \text{ total water} = (\text{wet wt} - \text{dry wt}) / \text{wet wt} \times 100.$$

Since there was no significant difference between the wet and dry weights of the control and reserpine-treated muscles, the data were pooled and averaged. The average wet weight of 49 muscles was 6.43 ± 0.78 (SD) mg while the average dry weight was 1.68 ± 0.34 mg.

Reserpine (1 mg/kg) was administered intraperitoneally either 4 or 24 hr prior to the experiment.

The standard error of the mean is indicated after each average value and the Student t test was employed to determine significance of the difference between means. p values of less than .05 indicate statistical significance.

Results. A. Total potassium, sodium, and water content of papillary muscles. The water content of 6 papillary muscles before immersion in Krebs–Ringer solution was on the average 74.5 ± 0.26% total H_2O (Table I). After 320, 480, 510, and 650 min of immersion, the total water content increased to 77.1 ± 0.23, 77.2 ± 0.55, 78.1 ± 0.17, and 79.4 ± 0.44, respectively ($p < .001$); the longer the incubation period the greater the increase in water content ($p < .05$). In papillary muscles removed from cats treated with reserpine 4 or 24 hr prior

TABLE I. Total Water, Sodium, and Potassium Content of Cat Papillary Muscles.[f]

Period of immersion (min)	Reserpine pretreatment (hr)	No. of muscles	H_2O content (% total $H_2O \pm$ SE)	K content (meq/liter of tissue $H_2O \pm$ SE)	Na content (meq/liter of tissue $H_2O \pm$ SE)
0	0	6	74.5 ± 0.26[e]	98.2 ± 1.8[e]	53.7 ± 1.9[e]
320[a]	0	7	77.1 ± 0.23		81.6 ± 0.88
	4	3	77.1 ± 0.19		83.7 ± 1.23
	24	3	76.9 ± 0.30		81.3 ± 0.82
480[b]	0	5	77.2 ± 0.55	87.5 ± 2.2	—
	4	—	—	—	
	24	5	78.0 ± 0.31	84.7 ± 1.2	
510[c]	0	4	78.1 ± 0.17	—	61.0 ± 1.4
	4	—	—	—	—
	24	4	78.3 ± 0.13	—	59.1 ± 0.92
650[d]	0	6	79.4 ± 0.44	66.0 ± 1.6	
	4	4	78.7 ± 0.69	66.6 ± 1.9	
	24	5	78.5 ± 0.50	65.8 ± 1.4	

[a] Represents 120 min of equilibration with Krebs–Ringer solution and 200 min of incubation with ^{22}Na.

[b] Represents 120 min of equilibration with Krebs–Ringer solution and 360 min of incubation with ^{42}K.

[c] Represents 120 min of equilibration with Krebs–Ringer solution, 180 min of incubation with ^{22}Na, and 210 min of Na efflux (see Methods for details).

[d] Represents 120 min of equilibration with Krebs–Ringer solution, 360 min of incubation with ^{42}K, and 170 min of ^{42}K efflux (see Methods for details).

[e] The mean value is significantly different from all others listed in the same column ($p < .05$).

[f] Experiments were performed in cat papillary muscles stimulated at 60/min in a bath of Krebs–Ringer solution maintained at 37.5°.

to the experiment, the increase in water content after the same periods of incubation were similar to those found in the nonreserpine-treated muscles (Table I; $p > .05$).

While the total H_2O content increased during immersion in Krebs–Ringer solution, total potassium content of the muscles decreased. Thus, the potassium content of 6 papillary muscles prior to immersion was on the average 98.2 ± 1.8 meq/liter of tissue H_2O; after immersion in Krebs–Ringer solution for 480 and 650 min the potassium content significantly decreased to 87.5 ± 2.2 and 66.0 ± 1.6 meq/liter of tissue H_2O, respectively (Table I). The greater loss of K from the muscles used in the ^{42}K efflux studies (650 min of immersion) is probably due to more frequent changes of the muscle bath and the longer duration of immersion. The potassium content of muscles immersed in the bath solution for 320 and 510 min were not determined since these experiments were concerned only with Na exchange. In papillary muscles removed from cats treated with reserpine 4 or 24 hr prior to the experiment, the decrease in potassium content after the same period of immersion was similar to that found in nonreserpine-treated muscles (Table I).

The total Na content of muscles bathed in Krebs–Ringer solution tended to increase. Thus, while the Na content of papillary muscles just removed from the heart was in 6 muscles on the average 53.7 ± 1.9 meq/liter of tissue H_2O, after 320 and 510 min of immersion the Na content significantly increased to 81.6 ± 0.88 and 61.0 ± 1.4 meq/liter of tissue H_2O, respectively ($p < .01$). The smaller gain in Na content after 510 min of incubation probably results from the fact that during the part of the immersion period (190 min) which was concerned with Na efflux measurements, the bath was changed every 15 min. In the studies in which the muscles were incubated for 320 min, the bath was changed only once when

the solution containing ^{22}Na was substituted for the nonradioactive bath solution. Rayner and Weatherall (12) reported that frequent changes of the bath leads to loss of Na from the tissue.

In papillary muscles taken from hearts pretreated with reserpine 4 or 24 hr prior to the experiment, increases in the Na content similar to those in untreated muscles were noted ($p > .05$, Table I).

B. The effect of reserpine on ^{42}K efflux. In the first 10-min period of efflux following 6 hr of incubation of control papillary muscles with ^{42}K, $3.53 \times 10^3 \pm 95.1$ cpm on the average appeared in the bath while in the last 10 min of efflux (170 min) only $1.62 \times 10^2 \pm 41.5$ cpm were noted (Fig. 1). After 170 min, the counts usually became too small to measure. In reserpine-treated muscles a similar pattern was observed both 4 and 24 hr after treatment. In fact, the efflux curves were almost identical to that of the control (Fig. 1). Indeed, at the end of efflux (170 min) the muscle counts were also of the same order of magnitude in untreated and reserpine-treated muscles; the muscle counts in the 6 control muscles were on the average 240.3 ± 11.2 cpm, while 4 and 24 hr after reserpine, they were in 4 muscles on the average 229.6 ± 8.1 and in 5 muscles on the average 233.8 ± 14.6 cpm, respectively ($p > .05$).

C. Effect of reserpine on ^{42}K influx. In the control experiments after 1 hr of incubation with ^{42}K, the muscle counts of 5 muscles were on the average $9.76 \times 10^2 \pm 52.3$ cpm; 5 hr later, it increased on the average to $2.57 \times 10^3 \pm 55.6$ cpm. After 6 hr of incubation with ^{42}K the cpm in muscles taken from cats pretreated with reserpine either 4 or 24 hr prior to the experiment were in 3 muscles on the average $2.52 \times 10^3 \pm 43.4$ and in 5 muscles $2.53 \times 10^3 \pm 140$ cpm, respectively. These counts are not significantly different from those of the control muscles after a similar period of exposure to ^{42}K ($p > .05$).

D. Effect of reserpine on ^{22}Na uptake. Four and 24 hr after reserpine, the uptake curves were similar to that of the control experiments (Fig. 2). In the first 20 min of incubation in the control series there was on

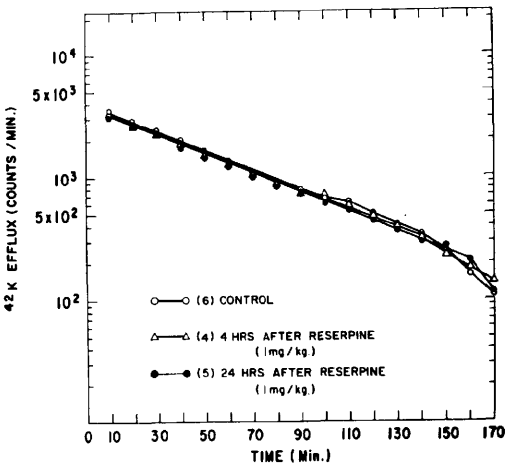

FIG. 1. The effect of reserpine on ^{42}K efflux. The muscles were loaded with ^{42}K during an incubation period of 6 hr. Fresh Krebs–Ringer solution was added to the muscle chamber when fluid was drawn off to measure radioactivity. Radioactivity of the solution was determined every 10 min (see Methods for details). The ordinate represents radioactivity of ^{42}K and refers to counts per minute appearing in the whole bath (10 ml). The number of observations in each series is indicated in parentheses. Reserpine (1 mg/kg) was administered 4 or 24 hr prior to the experiment.

the average a decrease of $1.37 \times 10^4 \pm 560$ cpm in the solution bathing the muscle. At the end of 200 min of incubation with ^{22}Na the total decrease in radioactivity of the solution was on the average $2.89 \times 10^4 \pm 1560$ cpm. Decreases in the counts were of a similar order of magnitude 4 and 24 hr after reserpine pretreatment (Fig. 2). In addition, the muscle counts at the end of the uptake studies were similar in the control and reserpine-treated series. Thus, 4 and 24 hr after reserpine they were on the average in 3 muscles 2208 ± 131 and 2231 ± 153 cpm, respectively, while in 7 control muscles they were 2148 ± 74.6 cpm ($p > .05$).

E. Effect of reserpine on ^{22}Na efflux. Reserpine given 24 hr prior to the experiment did not in any way affect Na efflux. Thus, after nonreserpine-treated muscles were incubated with ^{22}Na, in the first period of efflux (15 min) $2.94 \times 10^3 \pm 81.3$ cpm on the average appeared in the fluid bathing the muscle while in the last period of efflux (210 min) 200 ± 44.2 cpm appeared in the bath

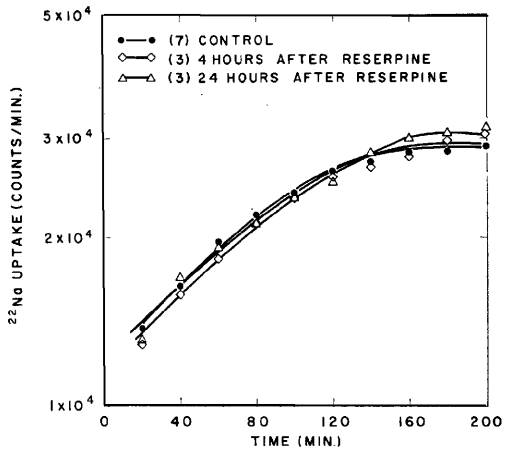

FIG. 2. The effect of reserpine pretreatment on ^{22}Na uptake. Reserpine (1 mg/kg) was administered intraperitoneally 4 or 24 hr prior to the experiment. Incubation with ^{22}Na started at 0 time. The number of observations is indicated in parentheses. The ordinate represents the cumulative decrease in the bath counts expressed as counts per minute. Radioactivity of the solution was determined every 20 min (see Methods for details).

(Fig. 3). In reserpine-treated muscles, similar levels were detected. Muscle counts at the end of efflux in 4 reserpine-pretreated muscles were on the average 124 $\pm$ 3.3 cpm while in 4 control muscles they were 127 $\pm$ 1.6 cpm($p > .05$).

Discussion. The results of these experiments indicate that reserpine administered either 4 or 24 hr prior to the experiment in a dose which is effective against digitalis-induced arrhythmias (2) does not affect Na and K exchange in isolated cat papillary muscles. In addition, changes occurring in total Na, K, and water content of the muscle during the course of the experiment were not influenced by reserpine pretreatment. In contrast, quinidine has been reported to inhibit K efflux and Na uptake and to increase and decrease K influx (5, 6, 13). In fact, under the same experimental conditions as in the present study, quinidine reduces K efflux and Na influx while it enhances K influx (Choi and Roberts, unpublished observations). There is also evidence which indicates that Na efflux is inhibited by quinidine (6). Furthermore, after quinidine the total K content of the muscle increases while total Na content decreases (13, 14). Consequently, the contention (4) that reserpine given either 4 or 24 hr prior to the experiment, antagonizes digitalis-induced arrhythmias by exerting a quinidine-like action on cardiac excitability is not supported by the results of the present investigation. The data however, are in accord with observations of Ciofalo *et al.* (15) that the antiarrhythmic spectrum of reserpine is more limited than that of quinidine and agrees with the results of Vaughan Williams (16) that unlike quinidine, pretreatment with reserpine does not cause changes in the transmembrane action potential of rabbit atria.

In a previous study using the same experimental conditions as in the present investigation, reserpine administered 24 hr prior to the experiment diminished the capacity of ouabain to induce arrhythmias in isolated papillary muscle (2). Since reserpine does not seem to produce quinidine-like effects in this preparation, it is suggested that the antiarrhythmic action of reserpine is related to the effect of the drug which depletes adrener-

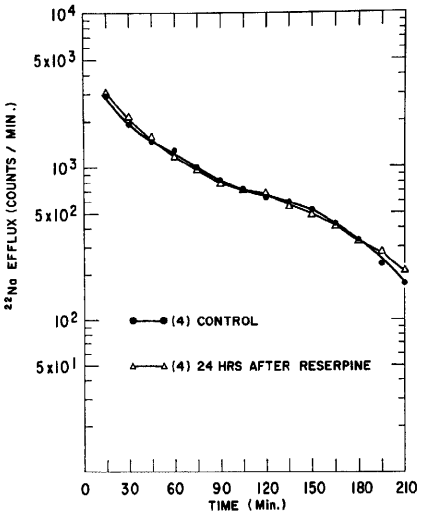

FIG. 3. Effect of reserpine on ^{22}Na efflux. The muscles were loaded with ^{22}Na for 3 hr before the efflux studies were initiated. Reserpine was administered 24 hr prior to the experiments. Radioactivity of the solution was measured every 15 min (see Methods for details). The ordinate represents the counts appearing in the solution (10 ml) bathing the muscle in counts per minute. The number of experiments is indicated in parentheses.

gic nerve terminals of the transmitter substance. Indeed, Roberts *et al.* (2) reported that the capacity of acetylstrophanthadin to induce arrhythmias is restored in reserpine-treated animals by the infusion of norepinephrine.

The loss of potassium, the gain of water and sodium when the muscles were immersed in the Krebs–Ringer solution confirms the observations of other investigators (11–13, 17). In addition, the values for Na and K content of papillary muscles immediately removed from the heart agree with those reported by Lee *et al.* (7).

Summary. Reserpine pretreatment (1 mg/kg) given either 4 or 24 hr prior to the experiment did not affect the transmembrane exchange of Na and K in cat papillary muscles. In addition, changes occurring in Na, K, and H_2O content of the muscle were not influenced by reserpine. Since it is known that quinidine causes changes in Na and K exchange and in the total Na and K content of cardiac muscle, it is unlikely that the action of reserpine to antagonize digitalis-induced ventricular arrhythmias is related to a quinidine-like effect on cardiac muscle.

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