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The effect of reserpine treatment and decentralization on the ion distribution in the vas deferens of the guinea-pig

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Summary

1. The ion distribution in the vas deferens of the guinea-pig was determined following reserpine treatment and decentralization, procedures known to increase the sensitivity of this muscle to drug stimulation.

2. There were no significant changes in the estimated intracellular ion concentrations following the two procedures. The half-times of ^{42}K and ^{36}Cl effluxes were also unaltered.

3. As far as ion content is concerned there is no basis for suggesting an altered resting membrane potential in supersensitive smooth muscle.

Introduction

Fleming (1963) proposed, on the basis of indirect evidence, that the development of nonspecific supersensitivity of smooth muscle, caused by decentralization or reserpine treatment, might change the properties of the cell membrane. Direct support implicating the cell membrane in this phenomenon was provided by Carrier & Shibata (1967), who demonstrated a substantial decrease in the calcium content of vascular smooth muscle after pretreatment with reserpine. Subsequently, Carrier, Douglas, Garrett & Whittington (1967) observed a decrease in sodium and potassium levels in vascular smooth muscle made supersensitive to noradrenaline by pretreatment with reserpine. These investigators suggested that the decrease in tissue calcium content may lead to an increase in permeability to sodium and potassium resulting in a partial depolarization and thus an increased excitability of the tissue to drug stimulation.

The present investigation was undertaken to determine whether such a mechanism could account for the nonspecific supersensitivity observed in another type of smooth muscle and further to determine whether changes in ionic content follow decentralization as well as reserpine treatment. The experiments were performed with the vas deferens of the guinea-pig, since this tissue exhibits a supersensitivity which is qualitatively and quantitatively similar following both decentralization and reserpine treatment (Westfall, 1970).

In contrast to the marked changes in ion content observed in vascular smooth muscle after reserpine, the electrolyte levels of the vas deferens were only slightly influenced by reserpine treatment or decentralization.

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Methods

Animals and tissue preparations

Guinea-pigs weighing between 300 and 500 g were randomly assigned to the following groups: (1) no treatment; (2) pretreatment with reserpine (1·0 mg/kg daily) for either 1 or 5 days; (3) animals in which both vasa deferentia were decentralized by hypogastric nerve section 1 or 5 days before the experiment. This treatment was selected because the vas deferens exhibits supersensitivity 5 days following either decentralization or daily reserpine treatment but not after only one day (Westfall, 1970).

Vasa deferentia were removed from freshly killed animals, quickly dissected free of connective tissue and weighed on a torsion balance. In one series of experiments, the tissues were not incubated in Krebs solution in order to provide an estimate of the ion content *in vivo*. In other experiments the tissues were incubated before ion analysis. For the latter experiments the muscles were mounted on a stainless steel holder as far as possible in a uniform condition by adjusting the ratio of weight (mg) to length (mm) to approximately 2·0 (Bülbring & Kuriyama, 1963). The tissues were left to equilibrate in Krebs solution at 37° C for at least one hour. At the end of the incubation period the tissues were placed on filter paper, covered with another filter paper and gently stroked. The wet weight was then determined and the tissues placed in a drying oven on small squares of Teflon tape. The tissues were dried for 16–20 h at 100° C, easily removed from the Teflon, re-weighed to obtain the dry weight and prepared for ion analysis. Teflon has the advantage that the tissue does not adhere to it and that it can be carried through the analysis without interfering with the ion determination.

Ion determinations

Ionic contents were determined by flame photometry with some modifications of the techniques of Casteels & Kuriyama (1965). The dried tissues, together with the Teflon tape, were placed in Vitreosil tubes with 1·5 ml hydrogen peroxide and the contents evaporated to dryness in an oven at 100° C. Concentrated $AgNO_3$ solution was added to the H_2O_2 to obtain a concentration of 1·6 μM. This caused the precipitation of tissue chloride as AgCl during the ashing procedure. The dried ash was dissolved in the test tube with 5 ml of a "diluting solution" containing 1 N HNO_3, 0·02 M H_3PO_4 and 4·6 mM Li_3CO_3. The Li was included in order to saturate the flame with cation and thus reduce cation interaction. The Na, K, Mg and excess Ag concentrations of the supernatant were determined by atomic absorption flame photometry at wavelengths of 587, 762, 285 and 328 mμ with a Unicam SP. 90 atomic absorption spectrophotometer. The Ca concentration was determined by emission at a wavelength of 424 mμ with a Zeiss spectrophotometer PMQII with flame attachment. The ion concentrations in the test solutions were compared with standards containing equal concentrations of Na and K and equal concentrations of Ca and Mg, the Ca and Mg concentrations being one-tenth of the Na and K concentrations. The Ag standards were separate.

Extracellular space

The extracellular space was determined by employing [^{60}Co] EDTA as an extracellular marker (Brading & Jones, 1969). After equilibration in normal Krebs

solution the tissues were transferred for 15 min to Krebs solution containing [^{60}Co] EDTA, 1–2 μCi/ml with CoEDTA of 0·1 mM. After the ions in the tissues and standards had been extracted into the diluting fluid in preparation for ion analysis but before flame photometry, the [^{60}Co] EDTA activity in each testtube was measured in a gamma well scintillation counter. Counting was therefore conducted without interference with the measurements of ions. This method made it possible to determine the wet weight, dry weight, extracellular space and ion content in each individual tissue.

^{42}K and ^{36}Cl effluxes

For these experiments, freshly dissected tissues were mounted and loaded in Krebs solution at 37° C in which either ^{42}KCl was substituted for KCl or Na ^{36}Cl was substituted for part of the NaCl. Loading time was 3 h for ^{42}K and 1 h for ^{36}Cl. After loading, the muscle was transferred every 2 min for 1 h through a series of testtubes containing 5 ml non-radioactive Krebs solution. For the ^{42}K experiments the amount of radioactivity in each tube was counted directly in a gamma well scintillation counter. For ^{36}Cl the activity was determined by liquid scintillation counting. To obtain the tissue counts the ^{36}Cl was extracted into distilled water. Details for the calculation of the efflux results have been described by Brading, Bülbring & Tomita (1969).

Solutions and statistical analysis

The Krebs solution used throughout this study was aerated with 97% oxygen/3% carbon dioxide and had the following composition (mM): Na^+ 137 ; K^+ 5·9 ; Ca^{++} 2·5 ; Mg^{++} 1·2 ; Cl^- 134 ; HCO_3^- 15·5 ; $H_2PO_4^-$ 1·2 ; glucose 11·5.

Statistical comparisons of the various parameters were always made among tissues taken from animals of the same batch. Differences between mean values were tested for significance by Student's *t* test according to Snedecor (1956). The 0·05 level of probability was considered significant.

Results

The effect of reserpine treatment and decentralization on the extracellular space, water and ion contents are shown in Table 1. The total tissue ions are expressed as mmol/kg wet weight. The total tissue water content was calculated from the values for wet and dry weight of the tissue. The value for the intracellular water content was obtained from the wet and dry weight plus the extracellular space.

After only 1 day of reserpine treatment or 1 day following decentralization there were no significant differences from the control in any of the parameters. When the treatments were extended to 5 days, several differences appeared. Tissues from both experimental groups showed a slight but significant increase in dry weight/wet weight ratio and a decrease in the total tissue water content. There was a significant elevation in the Mg content in tissues from reserpine-treated animals and an increase in Ca content in decentralized vas deferens.

Table 1 also shows the estimated intracellular concentrations of sodium, potassium and chloride in mmol/l cell H_2O. These values were calculated from the total tissue ion values and the extracellular space measurements. None of the treatments significantly altered these values.

TABLE 1. *Mean values* (±S.E.) *of water content, extracellular space, total and intracellular ion content of control, reserpine treated and decentralized vas deferens*

Treatment	Total							Dry weight/ Wet weight ×100	Cell			
	H_2O % wet weight	^{60}Co EDTA % wet weight	Na	K	Cl	Mg	Ca		H_2O % wet weight	Na	K	Cl
			mmol/kg wet weight							mmol/l cell H_2O		
None (control)	83·8	27·9	45·9	80·8	55·2	8·24	2·58	16·2	55·8	13·5	141·3	32·6
$n=10$	±0·1	±1·4	±0·8	±0·9	±1·3	±0·6	±0·09	±0·1	±1·5	±2·3	±2·3	±2·4
Reserpine (1 day)	83·6	27·7	48·4	78·8	55·7	9·28	2·57	16·4	55·8	18·3	135·9	33·1
$n=10$	±0·2	±1·2	±1·6	±1·1	±1·8	±1·0	±0·14	±0·2	±1·3	±2·4	±2·9	±3·5
Decentralization (1 day)	83·2	24·9	45·3	77·2	55·3	8·42	2·70	16·8	55·2	18·9	136·7	37·5
$n=10$	±0·3	±1·0	±0·8	±0·8	±1·0	±0·6	±0·11	±0·3	±1·0	±2·2	±3·3	±2·7
None (control)	83·9	30·3	54·6	77·9	65·7	8·80	3·04	16·0	53·6	24·6	143·8	47·4
$n=24$	±0·09	±1·2	±2·0	±1·0	±1·9	±0·31	±0·18	±0·09	±1·1	±3·0	±2·3	±3·2
Reserpine (5 days)	83·3*	31·7	53·9	78·1	67·1	10·87*	3·25	16·6*	51·7	20·6	147·8	47·9
$n=28$	±0·18	±0·7	±1·1	±0·7	±0·7	±0·55	±0·29	±0·17	±0·6	±1·7	±1·6	±1·4
Decentralization (5 days)	82·9*	31·3	51·5	77·0	65·5	9·52	4·21*	17·0*	51·7	17·6	145·8	45·7
$n=12$	±0·25	±1·1	±1·5	±0·7	±0·7	±0·66	±0·68	±0·25	±1·2	±1·6	±2·6	±1·4

* Significantly different from control ($P<0·05$). *n*, Number of tissues.

TABLE 2. *Mean values* (±S.E.) *of total tissue ion content* (*mmol/kg dry weight*) *of control, reserpine treated and decentralized vas deferens in* (*A*) *incubated tissues and* (*B*) *non-incubated tissues*

Treatment	Na	K	Cl	Mg	Ca
(A)					
None (control) $n=10$	282·9±4·9	486·1±6·2	340·9±8·5	50·8±4·0	15·9±0·5
Reserpine (1 day) $n=10$	295·1±5·2	470·8±8·2	339·6±2·1	56·3±5·7	15·6±0·8
Decentralization (1 day) $n=10$	268·6±5·4	478·9±5·2	327·5±5·7	49·9±3·7	15·9±0·6
None (control) $n=24$	339·7±13·6	485·6±5·4	418·2±14·4	54·9±1·9	18·9±1·1
Reserpine (5 days) $n=28$	325·6±9·3	496·5*±5·1	404·4±7·1	65·8*±3·7	19·7±1·8
Decentralization (5 days) $n=12$	301·2±7·4	451·2*±4·3	383·7±4·4	55·7±3·7	24·6±3·9
(B)					
None (control) $n=8$	269·8±5·3	507·6±4·4	338·2±6·7	34·9±0·5	7·8±1·2
Reserpine (5 days) $n=6$	269·7±4·6	458·1*±8·1	345·7±8·4	35·4±0·3	7·6±1·3
Decentralization (5 days) $n=8$	283·1±7·8	470·3*±10·7	350·8±13·3	34·7±0·4	6·1±0·8

* Significantly different from control ($P<0·05$). *n*, Number of tissues.

These results clearly differ from those obtained in vascular smooth muscle (Carrier & Shibata, 1967; Carrier *et al.*, 1967). However, the ion contents in vascular smooth muscle were expressed on the basis of dry weight rather than wet weight. For this reason the results presented in Table 1 were recalculated on the basis of dry weight and are shown in Table 2A. Tissues obtained from animals treated for 1 day did not differ from the control. After reserpine treatment for 5 days there was a significant increase in total tissue magnesium. In addition, following 5 days of either treatment there was a small but significant reduction in potassium content. The sodium and chloride levels decreased to the same extent as potassium but were not significantly different from the control.

Table 2B shows the ion contents of nonincubated tissues. Chronic decentralization or reserpine treatment did not produce any changes in Na, Cl, Mg or Ca concentrations, whereas the K content was significantly reduced.

In an effort to determine whether the change in potassium content represented an altered permeability of the vas deferens to this ion, the efflux of ^{42}K was investigated. The results presented in Table 3 show that the half-time of the exponential phase of ^{42}K efflux was not significantly altered by decentralization or by reserpine. Similarly, reserpine treatment did not alter the Cl permeability, since the half-time of the exponential phase of ^{36}Cl-efflux in tissues after 5 days of reserpine treatment was not significantly different from that in normal tissues (Table 3). The half times of ^{42}K and ^{36}Cl efflux as well as the estimated intracellular ion concentrations are in good agreement with the results of Casteels & Ostyn (1968).

Discussion

The supersensitivity of the vas deferens to drug stimulation which occurs following 5 days' decentralization or reserpine treatment is not accompanied by a detectable change of the estimated intracellular ion content.

Carrier & Shibata (1967) observed a marked reduction in the total Ca content of vascular smooth muscle after reserpine treatment. The Ca loss varied from 20 to 60%, depending on the particular blood vessel, species and schedule of reserpine treatment. A subsequent report from the same laboratory demonstrated that reserpine treatment also substantially reduced the Na and K content (Carrier *et al.*, 1967). These investigators suggested that the decrease in tissue Ca following reserpine treatment may lead to an increased permeability to Na and K, resulting in a partial depolarization and thus an increased excitability of the tissue.

The results of the present experiments indicate that such a suggestion is inadequate to explain the supersensitivity of the isolated vas deferens which follows

TABLE 3. *Mean half-times (±S.E.) of the exponential phase of ^{42}K and ^{36}Cl effluxes in control, 5-day reserpine treated and 5-day decentralized vas deferens*

	$t\frac{1}{2}$(min)	
Treatment	^{42}K	^{36}Cl
Control	131±7·5 *n*=14	10·2±0·6 *n*=8
Reserpine	121±6·8 *n*=8	10·6±0·5 *n*=9
Decentralization	126±10·7 *n*=6	

n, Number of experiments.

reserpine treatment or decentralization. No loss of total tissue Ca was observed. In addition, there was no significant reduction in the Na content of the tissue. A slight but significant reduction of the total K content was observed if the concentration was expressed on the basis of tissue dry weight. The maximum K loss following 5 day reserpine treatment was only 10%. The decreased K content may merely reflect a change in water content of the tissue, since the value is not different from the control if expressed on the basis of tissue wet weight. The water content of the vas deferens was significantly reduced by both chronic reserpinization and decentralization. This is not surprising in view of the fact that animals subjected to either procedure suffered a loss in total body weight over the course of 5 days.

A correlation between the reduction in K content and the development of supersensitivity is doubtful. The supersensitivity was observed *in vitro* (Westfall, 1970). In the same condition, *in vitro,* when there was a significant reduction in K content there was also a decrease in the Na and Cl content. The reduction in Na and Cl, while not significant, was nevertheless of the same order of magnitude as the K loss. The result is that the relative proportions of K, Na and Cl remained constant. This observation, coupled with the finding that the estimated intracellular K content of supersensitive vas deferens is not different from the control indicates that there is, as far as ion content is concerned, no basis for suggesting an altered resting membrane potential.

The discrepancy between the ion-depleting effects of reserpine in vascular smooth muscle and vas deferens may be due to the doses of reserpine. With one exception, the doses employed by Carrier & Shibata (1967) were 10–20 times higher than that found to be optimal for producing supersensitivity in vascular smooth muscle (Hudgins & Fleming, 1966) and 3–5 times higher than that of the present experiments. The dose of reserpine used in the current investigation is the lowest known to produce a supersensitivity of the vas deferens and one which produces a change in sensitivity which is quantitatively the same as that produced by decentralization (Westfall, 1970).

The lack of significant ion changes in the vas deferens after reserpine treatment or decentralization does not negate the possibility that there is some alteration at the level of the cell membrane. For example, Casteels & Kuriyama (1965) and Bülbring, Casteels & Kuriyama (1968) were unable to detect changes in the ion content of the rat and guinea-pig myometrium during pregnancy while there were marked changes in the resting membrane potential, action potential amplitude, threshold for excitation and sensitivity to adrenaline. Similar changes could occur in the supersensitive vas deferens without a change in ion content or ion efflux. Furthermore, in the preceding paper, it was suggested that supersensitivity may result from an increased efficiency in the drug-induced mobilization of membrane-bound calcium. Such an alteration in the physiological state of the cells would not necessarily be accompanied by noticeable changes in ion content. Experiments are in progress to examine these possibilities.

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