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RESERPINE HAS A DIRECT ACTION AS A CALCIUM ANTAGONIST ON MAMMALIAN SMOOTH MUSCLE CELLS

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SUMMARY

1. The effects of reserpine on excitation–contraction coupling and ^{45}Ca exchange of smooth muscle cells of the rabbit ear artery and the guinea-pig taenia coli have been studied.

2. Reserpine inhibited the spontaneous mechanical activity of the taenia coli and the force development induced by 59 mM-external K or 10^{-5} M-carbachol. In the ear artery reserpine blocked the K-induced contraction but its effect on the contraction elicited by noradrenaline was smaller. At 0·2 mM-Ca, the inhibition of the tonic component of the noradrenaline-induced contraction was more pronounced than that of the phasic component.

3. This reserpine action was fully reversible for the noradrenaline stimulus in the ear artery but less so for K-induced contractions. The inhibitory action on contractions induced in taenia coli by K-rich solution and by carbachol was even less reversible.

4. The analysis of the effect of reserpine on the ^{45}Ca exchange in the ear artery has revealed that it inhibits the increase of the fractional loss induced by K depolarization, but that it does not exert a significant effect on the increased fractional loss induced by 10^{-5} M-noradrenaline.

5. Reserpine slows down the filling with ^{45}Ca of the agonist-sensitive store without affecting the steady-state amount of Ca taken up by the store.

6. A study of the degree of filling of the store by measuring the force development and the ^{45}Ca release elicited by noradrenaline in Ca-free medium, reveals that the force development after loading in a reserpine-containing medium remains less than the control, although the same amount of Ca is released from the store.

7. It was shown by using tetrabenazine that the inhibitory action of reserpine on the Ca exchange and the force development is not due to an interaction of reserpine with the receptor molecules that are responsible for its depleting action on aminergic granules.

8. These results strongly suggest that reserpine exerts a Ca antagonistic action on smooth muscle whereby it blocks the potential-dependent channels. However, reserpine also affects the receptor-operated channels to some extent and in addition at a high concentration it seems to exert an unspecific inhibitory action on the contractile system.

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INTRODUCTION

It is generally assumed that reserpine depletes stores of monoamine transmitter substances by blocking the uptake of amines into presynaptic storage granules (Shore, 1972). However, it is not widely recognized that reserpine also directly affects certain tissues apparently independently of its actions on the aminergic system.

Our attention was drawn to the direct effect of reserpine by a series of neuroendocrine studies *in vitro*. Reserpine, directly and selectively inhibited in a dose-dependent manner between 0·09 and 9·0 μM, the anterior pituitary secretion of prolactin without influencing synthesis (Login & MacLeod, 1981). Inhibition of prolactin release by reserpine occurred even in glands taken from animals depleted of amines by α-methyl-*p*-tyrosine, demonstrating the lack of involvement of the aminergic system in the direct inhibitory reserpine action (Login, Cronin, Lamberts, Valdenegro & MacLeod, 1982). The release of [^{3}H]dopamine induced by 50 mM-KCl from rat corpus striatum was blocked by 10 μM-reserpine (Dyck & Boulton 1980) and the K-induced secretion of rat anterior pituitary hormones was virtually abolished by simultaneous exposure to 9·0 μM-reserpine (Login, Judd, Thorner & MacLeod, 1982).

These diverse direct actions of reserpine could be due to an effect of this substance on the cytoplasmic Ca concentration. The present experiments were designed to evaluate this proposed action of reserpine.

Our knowledge of Ca movements in the ear artery of the rabbit (Droogmans, Raeymaekers & Casteels, 1977; Casteels & Droogmans, 1981) has provided us with a tissue in which the effect of reserpine on Ca exchange could be investigated. Reserpine was found to inhibit in this tissue the contractions induced by K depolarization and to reduce to a large extent the increase of ^{45}Ca uptake and ^{45}Ca exchange elicited by K depolarization. Because of these actions it is concluded that reserpine can be considered as a Ca entry blocker.

Preliminary communications on some of these results have been presented at the Oxford meeting of the Physiological Society (Casteels, Droogmans & Login, 1983) and at the meeting of the Belgian Physiological Society (Login, Droogmans & Casteels, 1983).

METHODS

Rabbits (2–3 kg body weight) of both sexes were used in these experiments. The animals were stunned and bled. The ear artery was isolated and transferred to warmed and oxygenated Krebs solution. It was cleaned of its periarterial connective tissue under a dissection microscope and helical strips were cut for either contraction or flux experiments. The possible effects of released endogenous noradrenaline were eliminated by exposing the tissues for 20 min to a solution containing 300 mg/l. 6-hydroxydopamine at pH 4 (Aprigliano & Hermsmeyer, 1976). This procedure was then followed by an exposure to Krebs solution for 1 hr. Strips of taenia were dissected from the caecum of guinea-pigs and immediately transferred to warmed and oxygenated Krebs solution. The isometric force development of these two different tissues was studied by mounting them in a chamber of 1 ml. volume with a superfusion rate of 4 ml./min and attaching one end of the tissue to a force transducer.

For flux experiments the tissues were mounted isometrically on Teflon holders and allowed to equilibrate for 1 hr in Krebs solution. After exposing the tissues to^{45}Ca-containing solutions for the periods indicated in the results, the tissues were removed from the Teflon holders and mounted in a temperature-controlled perfusion chamber having a flow rate of 2 ml./min. The effluent was collected at 4 min intervals by automated fraction collection. At the end of the experiment the

tissues were blotted, weighed, ashed and dissolved. The rate of efflux, the tissue tracer content and the fractional loss were calculated from the radioactivity remaining in the tissue and that in the effluent samples.

The Krebs solution contained (mM): K, 5·9; Ca, 1·5; Mg, 1·2; Cl, 143·8; HEPES, 11·6; glucose, 11·5. It was bubbled with O_2 and the temperature was kept constant at 35 °C. Changes of $[K]_o$ were compensated by equivalent modifications of $[Na]_o$ to maintain constant osmolarity. In many experiments $[Ca]_o$ was reduced to 0·2 mM because the filling of the noradrenaline-sensitive store (Casteels & Droogmans, 1981) was more easily evaluated at this Ca concentration and because in ^{45}Ca-loading experiments a higher specific activity in the tissues could be obtained. Ca-free solutions always contained 2 mM-EGTA.

The following drugs were used at concentrations given in the results: noradrenaline (Fluka), reserpine (Sigma), carbachol (BDH) and tetrabenazine methanesulphonate, which was generously provided by Hoffman – La Roche Pharmaceutical Co. Reserpine was dissolved and diluted in dimethyl sulphoxide (DMSO) before adding it to the physiological solutions. In all experiments including reserpine the final concentration of DMSO was 0·5 %. In control experiments we observed that this DMSO concentration did not exert a significant effect on the parameters we examined. Statistical analysis was performed using Student's *t* test with significance accepted at the 0·05 level.

RESULTS

Action of reserpine on the force development induced by K depolarization or by an agonist

The spontaneous mechanical activity of taenia coli was inhibited by the addition of reserpine, as has been observed by Plummer, Earl, Schneider, Trapold & Barrett (1954) for the ileum. The force development elicited by 59 mM-external K was inhibited to about 50 % of the control by 1 μM-reserpine and to less than 10 % of the control by 9 μM (Fig. 1). Also the force development induced by 10^{-5} M-carbachol was diminished by reserpine to the same extent as the K-induced contractions.

Reserpine also inhibited the K-induced contraction of the rabbit ear artery in a manner very similar to that observed in taenia coli (Fig. 2). A second procedure to elicit contractions of the ear artery was the addition of noradrenaline to the bathing solution. Such contractions consist of an initial phasic response and an ensuing tonic component (Steinsland, Furchgott & Kirkepar, 1973). The initial response depends largely on the release of Ca from an intracellular store and it therefore also occurs in Ca-free medium. The tonic response requires a continuous supply of extracellular Ca delivered through receptor-operated channels (Bolton, 1979). Reserpine exerted a different inhibitory effect on these two components of the noradrenaline contraction. Fig. 2 shows the phasic and the tonic component of the force development of the ear artery induced by 10^{-5} M-noradrenaline in a solution containing 0·2 mM-Ca^{2+} and in the presence of different concentrations of reserpine. The tonic force development was inhibited to a much greater extent by reserpine than the phasic component. A similar pattern of inhibitory action by reserpine was also observed for stimuli with 10^{-7} and 10^{-6} M-noradrenaline. Both in the taenia coli and in the ear artery the inhibitory action of reserpine is appreciably reduced by increasing $[Ca]_o$. It was also important to evaluate the reversibility of these reserpine effects. We therefore studied the recovery of the force development of ear artery and taenia coli in reserpine-free medium after superfusion for 60 min with 3 μM-reserpine. These data are summarized in Table 1. In the ear artery the inhibition by reserpine is reversible for the noradrenaline stimulus, but not for the stimulation with K-rich medium. In contrast the inhibition by reserpine of the force development in taenia coli due to carbachol

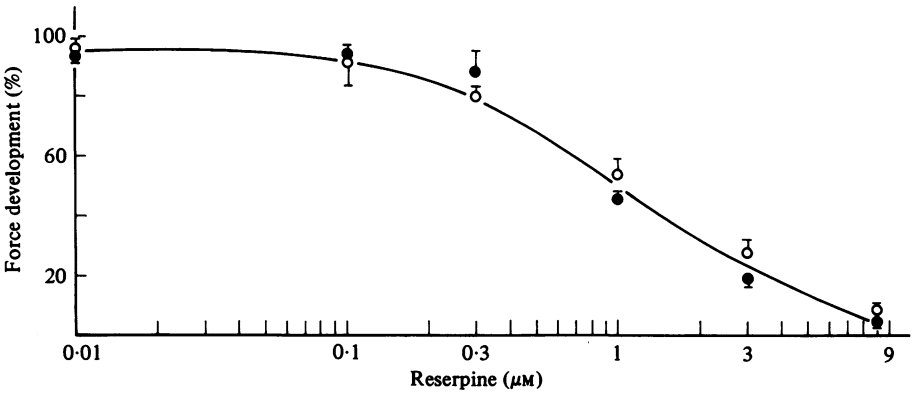

Fig. 1. Action of different concentrations of reserpine on the force development of the taenia coli of the guinea-pig induced at 0·2 mM-Ca by 59 mM-K (○) or by 10^{-5} M-carbachol (●) applied for 5 min. The tissues were superfused for 30 min with the reserpine-containing solution and the force development was determined in the presence of reserpine. The sequence of the different reserpine concentrations was from lower to higher concentrations. The reserpine concentrations are given on a logarithmic scale on the abscissa in micromolar and the force development as a percentage of the control before addition of reserpine. The experimental values are mean ±S.E. of mean for measurements on four tissues.

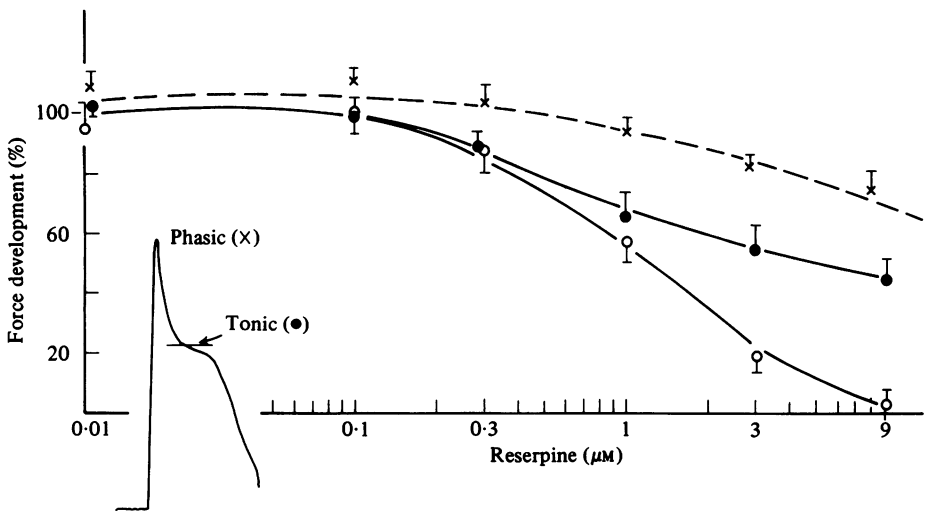

Fig. 2. Action of different concentrations of reserpine (μM), as indicated on a logarithmic scale on the abscissa, on the force development of the rabbit ear artery induced in solutions containing 0·2 mM-Ca by either 59 mM-K (○) or by 10^{-5} M-noradrenaline applied for 5 min. We have plotted the response to noradrenaline as the phasic (×) and the tonic component (●) which were measured as indicated in the inset. The tissues were superfused for 30 min with solutions containing increasing concentrations of reserpine. The force development is represented on the ordinate as a percentage of the control before addition of reserpine. The data are the mean values ±S.E. of mean of four tissues.

TABLE 1. Reversibility of the inhibitory action of reserpine on the force development of the ear artery and of the taenia coli

		10^{-5} M-noradrenaline		
	59 mM-K	Phasic	Tonic	10^{-5} M-carbachol
Ear artery				
60 min reserpine	26·2±4·6	78·9±7·5	48·6±7·8	—
60 min reserpine and 3 hr reserpine-free superfusion	67·5±10·3	106±7·8	100±5·6	—
Taenia coli				
60 min reserpine	15·3±5	—	—	7±3
60 min reserpine and 3 hr reserpine-free superfusion	23±10	—	—	44±12

Both tissues were stimulated with 59 mM-K^+ and either 10^{-5} M-noradrenaline (ear artery) or 10^{-5} M-carbachol (taenia coli). These stimuli were applied in a Krebs solution containing 0·2 mM-Ca^{2+} before addition of reserpine, after 60 min superfusion with 3 μM-reserpine and 3 hr after discontinuing the reserpine superfusion. The amplitude of the force development is expressed as a percentage of the response measured before application of reserpine. The values are given as mean ±S.E. of mean for four measurements.

stimulation or K depolarization was still very pronounced after 3 hr reserpine-free medium.

The inhibitory action of reserpine on the force development does not only depend on the concentration but also on the time of exposure. As shown in Fig. 3 the inhibition by 1 μM-reserpine of K-induced contractions in taenia coli and ear artery might have reached a steady state after an exposure *in vitro* exceeding 120 min. In contrast at 9 μM-reserpine a complete inhibition of the K-induced contraction was obtained after an exposure of 50 min.

Effect of reserpine on ^{45}Ca exchange

Because the above observations were reminiscent of the actions of Ca antagonists, we investigated the effect of reserpine on the Ca exchange in the ear artery of the rabbit because ^{45}Ca exchange and its functional significance in this tissue can be interpreted more easily than in the taenia coli of the guinea-pig. We have tried to determine the action of reserpine on the increase of the Ca permeability of the membrane induced by K depolarization, on the release of Ca from the intracellular Ca store by noradrenaline, and on the filling of this store with external Ca after its depletion.

Reserpine and ^{45}Ca-exchange during K-depolarization

Strips of ear artery were incubated for 3 hr in a solution containing ^{45}Ca after which they were rinsed for 130 min in non-radioactive solution to eliminate extracellular Ca and follow the Ca efflux. During the last 30 min of this rinsing period the tissues were either exposed to the same solution, or to a solution containing 0·5% DMSO or one with 9 μM-reserpine dissolved in the same DMSO concentration. Neither of these two treatments altered the steady-state ^{45}Ca efflux. After 130 min of efflux, $[K]_o$

was increased from 5·9 to 59 mM. This change increased the fractional loss of ^{45}Ca in the control and the DMSO-treated tissues but in the reserpine-treated tissues no significant increase of the fractional loss was observed (Fig. 4*A*). This observation fits the hypothesis that reserpine might block the Ca channels which are opened by K depolarization. To study this issue more directly we investigated the effect of reserpine on the K-induced Ca uptake. The tissues were pre-treated for 20 min in a solution with 0·5% DMSO or with 9 μM-reserpine in 0·5% DMSO. They were then transferred for 5 min to similar solutions containing 0·2 mM-^{45}Ca and either 5·9 or 59 mM-K. The amount of Ca which has entered the cellular compartment was

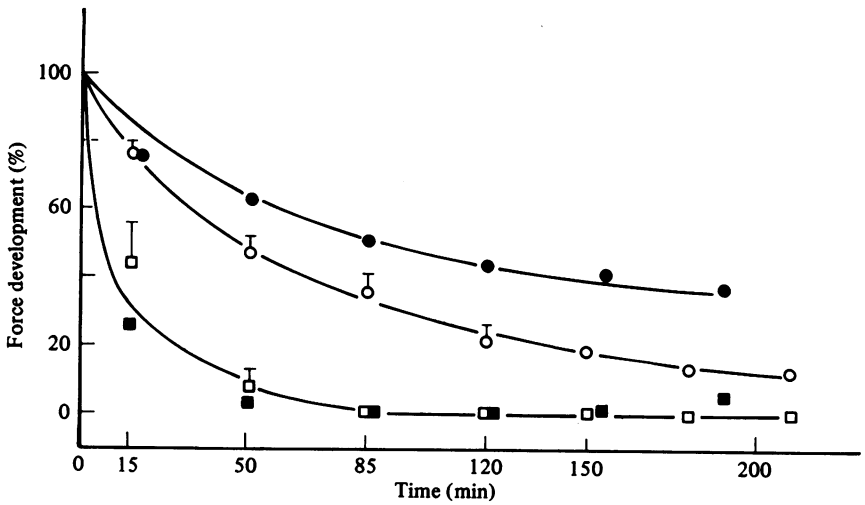

Fig. 3. Effect of the duration of exposure to a reserpine containing solution on the force development of taenia coli (open symbols) and of the ear artery (filled symbols) induced by 59 mM-K in a solution containing 1 mM-Ca. The force development is given on the ordinate as the percentage of the control value in the absence of reserpine. The time of exposure to either 1 μM-reserpine (circles) or 9 μM-reserpine (squares) is given on the abscissa. The values are mean values ±S.E. of mean of four determinations.

estimated by extrapolation from the subsequent efflux curve as described by Droogmans *et al.* (1977). These data are summarized in Table 2. Reserpine did not affect the Ca uptake in the control tissues, but it significantly reduced the stimulation of the Ca uptake by K-rich solution.

Reserpine and Ca exchange during stimulation with noradrenaline

In the ear artery exposed to Ca-free medium noradrenaline induces a release of Ca from an intracellular Ca store, thereby causing a transient force development and Ca extrusion (Droogmans *et al.* 1977). In Ca-containing medium, the same process of mobilization of cellular Ca is occurring, but because of the continuous replenishment of this store by external Ca the force development is sustained. This pathway for continuous calcium replenishment of the noradrenaline-sensitive store probably corresponds for the ear artery to the receptor-operated channels (Casteels & Droogmans 1981). We found it essential therefore to study the effect of reserpine on

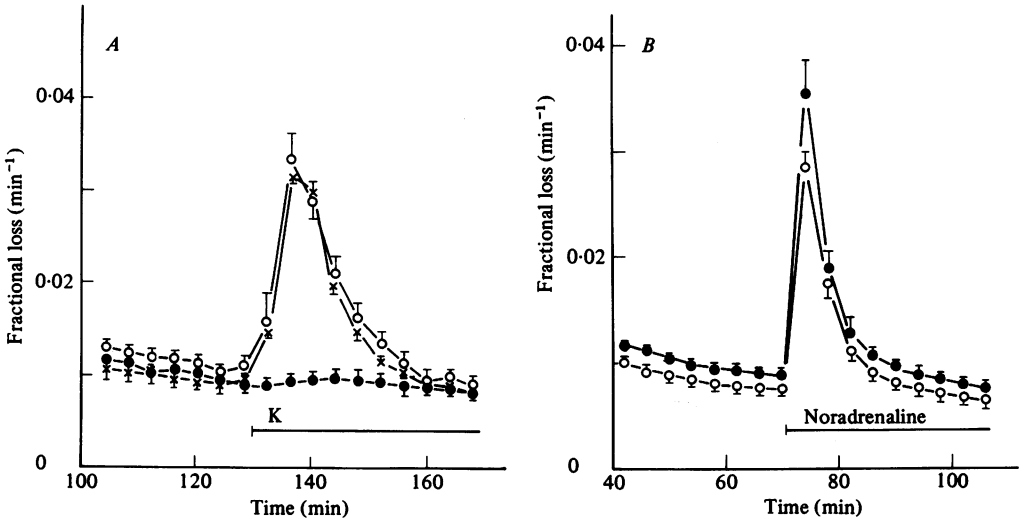

Fig. 4. Effect of 9 μM-reserpine on the fractional loss of ^{45}Ca induced by 59 mM-K or by 10^{-5} M-noradrenaline. Ear artery strips were loaded for 3 hr in a solution containing 0·2 mM-^{45}Ca. In *A* the tissues were first rinsed for 100 min in Krebs solution containing 5·9 mM-K and 0·2 mM-Ca, and then transferred for 30 min to the same solution (○) or to a solution with 0·5% DMSO added (×) or with 0·5% DMSO and 9 μM-reserpine (●). At 130 min of efflux, $[K]_o$ was increased from 5·9 to 59 mM. In *B*, a similar loading procedure was used but the tissues were then washed at 20 °C for 40 min in Ca-free medium and for another 30 min in either the same Ca-free medium (○) or in a Ca-free medium with 0·5% DMSO and 9 μM-reserpine added (●). After 70 min of efflux 10^{-5} M noradrenaline was added to the superfusion medium. The experimental data in *A* and *B* represent the mean values ±S.E. of mean of ten tissues.

TABLE 2. Effect of 9 μM-reserpine on the ^{45}Ca uptake (μmole/kg wet wt.) during K depolarization

Loading solution	^{45}Ca content (μmole/kg wet wt.)
5·9 mM-K	7·4±0·8 (6)
59 mM-K	31·4±2·1 (10)
5·9 mM-K+9 μM-reserpine	7·4±1 (6)
59 mM-K+9 μM-reserpine	16·2±1·9 (11)

The tissues were exposed for 20 min either to a solution containing 0·5% DMSO or 0·5% DMSO and 9 μM-reserpine before transferring them for 5 min to a similar solution containing 0·2 mM-^{45}Ca and either 5·9 or 59 mM-K. The amount of ^{45}Ca which was taken up by the tissue was estimated by extrapolating the subsequent washout curve representing the decrease of activity remaining in the tissue, back to zero time. This efflux lasted 120 min and was performed in a Ca-free solution at 20 °C. The number of tissues is given between brackets.

two properties of the intracellular Ca store i.e. release of Ca from this store by noradrenaline and refilling of the depleted store by external Ca.

As shown in Fig 4*B*, 9 μM-reserpine did not affect the fractional loss of ^{45}Ca induced by 10^{-5} M-noradrenaline in Ca-free medium. This absence of any effect implies that neither the release of Ca from this store, nor its subsequent extrusion out of the cells were affected by reserpine.

The influence of reserpine on the refilling of the store was studied by estimating the amount of Ca which entered the store following its depletion. Such Ca-depleted tissues were obtained by stimulating them with 10^{-5} M-noradrenaline in Ca-free medium. Subsequent exposure of such tissues to a Ca-containing medium led to a rapid reaccumulation of Ca into the store. We estimated the effect of reserpine on the degree and rate of calcium reaccumulation in two ways i.e. by determining the amplitude of the phasic contraction and the amount of ^{45}Ca released by a noradrenaline

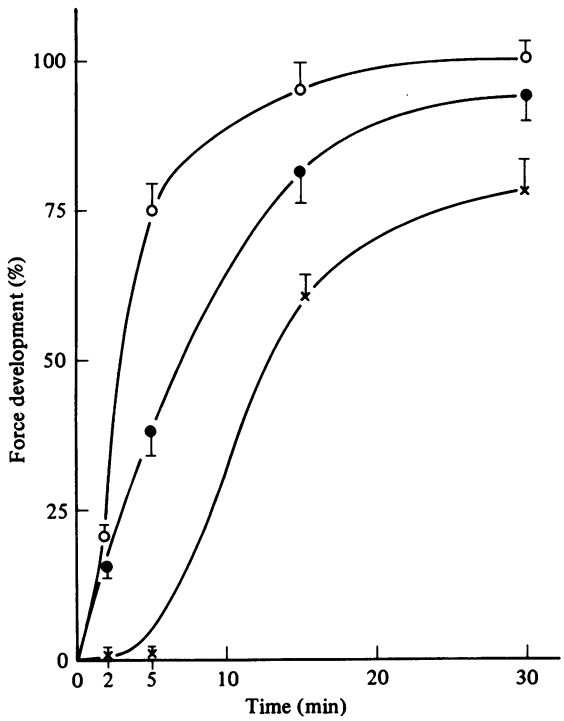

Fig. 5. Action of reserpine on the filling of the noradrenaline-sensitive Ca store as estimated by force development. Tissues were first depleted of Ca by stimulating with 10^{-5} M-noradrenaline in Ca-free medium. They were then exposed for a variable period of time as indicated on the abscissa to a solution containing 0·2 mM-Ca^{2+} (○) or to the same solution with 3 μM (●) or 9 μM (×) reserpine. They were then washed for 5 min with Ca-free medium and stimulated with 10^{-5} M-noradrenaline in this solution. The force development induced by 10^{-5} M-noradrenaline after 5 min exposure to Ca-free medium is given on the ordinate as a percentage of the control value determined after 30 min. There were fifteen tissues in the 3 μM-reserpine group and seven in the 9 μM group.

stimulus in Ca-free solution. The degree of filling of the store, as estimated by the force development induced in Ca-free medium by 10^{-5} M-noradrenaline is represented in Fig. 5. In the presence of 3 and 9 μM-reserpine the filling of the store with Ca proceeded more slowly than in the control solution as suggested by the significant difference ($P < 0·01$) between force development after the 5 min uptakes. However, the contraction after 15 and 30 min of filling was only slightly reduced for 3 μM-reserpine while for 9 μM-reserpine no contraction was observed for loading times

below 5 min and the contractions at 15 and 30 min were appreciably less than in the control (Fig. 5). These findings are in agreement with the observation (Fig. 2) that the inhibitory action of high concentrations of reserpine on the amplitude of the tonic response is more pronounced than that on the amplitude of the initial phasic response of the noradrenaline induced contraction.

The effects of 3 and 9 μM-reserpine on the ^{45}Ca uptake by tissues in which the noradrenaline-sensitive store was depleted are represented in Table 3. Both 3 and

TABLE 3. The action of 3 μM and 9 μM-reserpine on the ^{45}Ca uptake (μmole/kg wet wt.) by Ca-depleted tissues

Loading time (min)	Control	3 μM-reserpine	9 μM-reserpine
5	27·2 ± 4·3	16·1 ± 2·5	11·8 ± 1·5
30	46·1 ± 3·2	44·2 ± 2·8	46·3 ± 2·9

The tissues were pre-treated for 30 min with either 0·5 % DMSO or with 3 μM or 9 μM-reserpine in 0·5 % DMSO and depleted of Ca by stimulation with 10^{-5} M-noradrenaline in Ca-free medium. These depleted tissues were then exposed for 5 or 30 min to a loading solution with 0·2 mM-^{45}Ca in the absence of reserpine or in the presence of 3 μM or 9 μM-reserpine. The total amount of Ca accumulated in the tissues was estimated by extrapolating to time zero the curve representing the decrease of the ^{45}Ca remaining in the tissue during an 100 min efflux in Ca-free medium at 20 °C. There were six tissues in each group.

9 μM-reserpine reduce the amount of ^{45}Ca taken up during 5 min exposure to a solution containing ^{45}Ca. However for the 30 min uptakes, the values obtained in the presence of 3 and 9 μM-reserpine are not different from the control. Also the amount of ^{45}Ca released after filling the store for 30 min in a solution containing 0·2 mM-Ca is not affected by the presence of 9 μM-reserpine. At minute 60 of the efflux in Ca-free medium the amount of ^{45}Ca released from this store by 10^{-5} M-noradrenaline was 7·2 ± 1·3 μmole/kg wet weight in the control and 7·9 ± 1·1 μmole/kg wet weight after loading in the presence of 9 μM-reserpine. These mean values ± S.E. of mean for six tissues are not different from those given by Casteels & Droogmans (1981). It can therefore be concluded that the amount of Ca present in the agonist-sensitive store after 30 min is not modified by 9 μM-reserpine. It remains to be explained why the force development is appreciably reduced by the presence of 9 μM-reserpine.

Interaction of tetrabenazine and reserpine on K-induced contractions

Tetrabenazine, a weak and short acting neuroleptic, competes with reserpine for the same receptor molecules on aminergic granules (Giachetti & Shore, 1978). Because tetrabenazine is a competitive agonist of reserpine, pre-treatment with this substance can prevent the action of reserpine (Quinn, Shore & Brodie, 1959). We used this property to study whether the direct action of reserpine on Ca exchange was mediated by activation of this receptor.

Tissues were pre-treated for 30 min with a solution containing 10 μM-tetrabenazine. Neither the force development nor the Ca exchange elicited in the ear artery by 59 mM-K^+ were affected by this drug. Furthermore addition of 3 μM-reserpine to the tetrabenazine-containing solution induced the same inhibitory action on K-induced force development and ^{45}Ca exchange as in the absence of tetrabenazine. These

observations support the hypothesis that the direct inhibitory action of reserpine on denervated smooth muscle does not depend on the same receptor mechanism which is responsible for its depleting action on the amine stores.

DISCUSSION

The best known action of reserpine is the depletion of stores of catecholamines and 5-hydroxytryptamine which depends on interaction of reserpine with a specific receptor molecule on the presynaptic storage granules (Giachetti & Shore, 1978). However since the earliest investigations of the physiological and pharmacological actions of reserpine, some effects of this substance could not be explained by its influence on the amine stores. Besides the findings of Plummer *et al.* (1954) on the isolated rabbit ileum we have to mention the observations of Zonta, D'Agostino, Lucchelli & Grana (1976). These authors found that 1·6–3·2 μM-reserpine diminished the force development of the rat vas deferens elicited by exogenous catecholamines. In heart muscle reserpine directly depressed contractility (Tripod & Meier, 1954) and lowered the pace-maker rate independently of the adrenergic system (Brimijoin & Trendelenburg, 1971). Nayler (1963) confirmed the negative inotropic action of reserpine in toad ventricular muscle and showed that the decline in contractility could be rapidly overcome by increasing the external Ca concentration. All these actions and those described in relation to the pituitary gland suggest that reserpine might affect the permeation of Ca across the cell membrane. The use of tetrabenazine, which is a short-acting competitive agonist at the reserpine receptor has allowed us to demonstrate that the Ca antagonistic action of reserpine does not depend on the activation of the same reserpine receptor which is responsible for the blockade of the amine uptake into presynaptic storage granules. Our observations on the modification by reserpine of the force development and ^{45}Ca exchange in the smooth muscle of the ear artery support this hypothesis. The inhibition by reserpine of the force development and of the ^{45}Ca exchange induced by K depolarization is similar to that obtained by Ca antagonists such as D600 and nicardipine. (Casteels & Droogmans, 1981). The potential-dependent pores for Ca which are opened by K depolarization (Bolton, 1979) seem to be as much affected by reserpine as by the usual Ca entry blockers. However, reserpine was also found to inhibit to some extent the force development elicited by noradrenaline. A study of the ^{45}Ca uptake by the agonist-sensitive store has revealed that the rate of filling of this store is reduced by reserpine and this inhibition is larger for the higher reserpine concentration. According to the findings of Casteels & Droogmans (1981) and of Casteels, Droogmans, Raeymaekers & Wuytack (1981) this rate of filling would depend on the opening of the receptor-operated channels. Because noradrenaline concentrations below 10^{-6} M do not depolarize the ear artery cells (Droogmans *et al.* 1977) and because the force development elicited by such noradrenaline concentrations is similarly reduced by reserpine, it can be assumed that reserpine also acts at the receptor-operated channels. The above findings therefore suggest that reserpine might be less specific than other Ca entry blockers and that it exerts an action on the potential-dependent pores and on the receptor-operated channels. This wider action of reserpine could explain why the inhibition by reserpine of the tonic component of the noradrenaline-

induced contraction is larger than that of the phasic component. However according to our findings there is no decrease of the total amount of ^{45}Ca taken up by the agonist-sensitive store after 30 min, while the force development induced by noradrenaline in Ca-free medium remains appreciably smaller in the presence of 9 μM-reserpine than in the control. Because in both conditions the same amount of Ca becomes available for activation of contraction, we have to assume that either reserpine is slowing down the rate of release of Ca from this store, or that it exerts an action not directly related to the availability of Ca, e.g. by interacting with calmodulin. However this possible third mechanism of action of reserpine is only obvious at the higher reserpine concentration.

The above findings also suggest that the effects of reserpine administration *in vivo* may be even more complex than currently accepted. For example, it is possible that Ca antagonism is responsible for the generalized exocrinopathy observed during reserpine administration to animals (Martinez, Quissell, Wood & Giles, 1975; Perlmutter & Martinez, 1978). The same action of reserpine could also induce the arterial vasodilation following its intra-arterial injection in the management of Raynaud's phenomenon in humans (de la Lande, Parak, Landrian, Skinner & Whelan, 1960; Wilkeron, Thompson, Hookman, Herdt & Decker, 1970). In the future it will be important to differentiate between indirect reserpine effects related to amine depletion and direct reserpine actions as a Ca antagonist.

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