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A COMPARISON OF RATES OF DEPLETION AND RECOVERY OF NORADRENALINE STORES OF PERIPHERAL AND CENTRAL NORADRENERGIC NEURONES AFTER RESERPINE ADMINISTRATION: IMPORTANCE OF NEURONAL ACTIVITY

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1 The noradrenaline (NA) content of various peripheral sympathetic neuroeffector organs and brain was measured at various times after different doses of reserpine administered to the rat.

2 About a 25% reduction in the NA content of the heart was observed 24 h after 0.005 mg/kg reserpine. Two to ten times more reserpine was needed to obtain an approximately similar degree of depletion in the salivary gland and vas deferens; 0.1 mg/kg produced almost complete depletion in the heart and salivary gland, whereas 5 mg/kg was needed to deplete the vas deferens of its NA content.

3 The NA content of the brain and superior cervical ganglion was lowered by 20 to 30% in 24 h only when the reserpine dose was raised to 0.1 mg/kg, and 5 to 10 mg/kg was required to obtain over 95% depletion.

4 The rates of recovery of cardiac and salivary gland NA stores, after about 80 to 100% depletion by low and high doses of reserpine, were almost identical. About 50% restoration occurred in 7 to 15 days after a single dose of 0.1 mg/kg reserpine.

5 The superior cervical ganglion, the NA content of which was fully depleted by 10 mg/kg, showed almost complete recovery in about 7 days.

6 Transmural stimulation of the left atrium of the guinea-pig for 30 min (5 Hz for 30 s/min), or exposure of the atrium to reserpine (5 μg/ml) for 30 min, caused modest but statistically insignificant reduction in tissue NA content. However, stimulation in the presence of reserpine 5 μg/ml for 30 min produced about 50% depletion of NA.

7 *In vitro* reduction in NA content caused by reserpine plus transmural stimulation, was even more pronounced after treatment of the isolated vas deferens of the rat with tetraethylammonium.

8 It is suggested that different rates of depletion following *in vivo* administration of reserpine are mainly due to variation in neuronal activity of different sympathetic neuroeffector organs.

Introduction

Reserpine is well known for its depleting effect on noradrenaline (NA) stores of the peripheral and central noradrenergic neurones. The exact mechanism(s) by which depletion and restoration of NA stores occurs after reserpine treatment remains obscure at the present time.

The purpose of the present investigation was threefold. The first was to study the effect of very low doses of reserpine, comparable to those used in therapeutics, on the NA content of various peripheral sympathetic organs and of whole brain. The second was to compare the rates of recovery of NA content following an equivalent depletion by 'low' and 'high' doses of reserpine. The third was to see if the NA content of the isolated sympathetic organs can be depleted *in vitro* by reserpine. A preliminary account of some of these findings has already appeared (Wakade, Rosenberg & Mark, 1976; Wakade, 1978a).

Methods

Removal of tissues

In some experiments male rats (300 to 400 g) were killed by a blow on the head, and the submaxillary gland, heart and vas deferens were removed. In other experiments rats were anaesthetized with ether in order to remove both superior cervical ganglia and the whole brain. Male guinea-pigs (400 to 500 g) were killed by a blow on the head, and the heart was removed. The dissection of these tissues was carried

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out in oxygenated Krebs solution. In the case of the salivary gland, the sublingual gland (which is located on the top portion of the submandibular gland and has practically no sympathetic innervation) was gently separated and then removed. The submandibular gland was used in the present study, and has been referred to as the salivary gland. The left atrium was dissected away from the whole heart of the guinea-pig and split in half, as described earlier (Wakade & Furchgott, 1968).

Transmural stimulation

Left atrial halves and vas deferens were placed between two platinum plate electrodes mounted in a leucite holder for the purpose of transmural stimulation. Each tissue was secured to the bottom hook of the holder by means of a cotton thread. The other end of the tissue was tied by a thread and attached to the upper hook to hold the tissue firmly between the plate electrodes. The holder, along with the tissue, was placed in a cylindrical muscle chamber containing 20 ml Krebs solution at 37°C, through which 95% O_2 and 5% CO_2 was continuously bubbled. A train of 150 impulses at 5 Hz was delivered for 30 s every min for 30 min or 2 h (voltage dial set at 80; 1 ms duration) from a Grass stimulator, Model S-88. Appropriate concentrations of reserpine phosphate (Ciba Pharmaceutical Company, Summit, NJ) or tetraethylammonium chloride (TEA) (Eastman Kodak Co., Rochester, NY) were added to the muscle chamber fluid 15 min before the beginning of the stimulation period.

Extraction and analysis

The salivary gland, heart, left atrium, vas deferens and brain were rapidly blotted and weighed, and transferred to a 20 ml plastic tube containing 5 ml of ice-cold 0.4 N perchloric acid. Tissues were homogenized for 30 s with a Polytron homogenizer (Brinkman Instruments). In the case of the superior cervical ganglia, right ganglia from two rats were pooled, washed, and transferred to a 3 ml glass homogenizer containing 1 ml of 0.4 N perchloric acid and homogenized at about 4°C with a glass pestle rotated by an electric motor at 600 rev/min. The homogenate was transferred to a centrifuge tube, and the pestle and homogenizer were washed with 0.4 N perchloric acid, with the wash fluid being added to the centrifuge tube. Tissue homogenates were centrifuged, and the supernatant was analyzed for NA by the method of Shellenberger & Gordon (1971). In all cases, standard solutions of NA were analyzed concurrently, with recovery ranging from 70 to 90%. Appropriate corrections for recovery were made for each sample, and the NA concentration was expressed in terms of free base. Mean values are given with standard errors. Student's

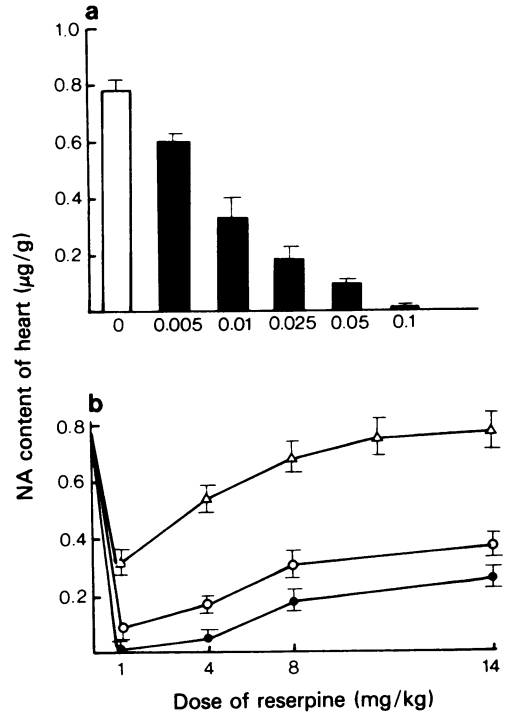

Figure 1 Effect of various doses of reserpine on the depletion and recovery of noradrenaline (NA) stores of the heart. Rats were injected with various doses of reserpine as shown (a), and their hearts were removed after 24 h for NA analysis. In another group (b), rats were injected with 0.01 (△), 0.1 (○), and 10 (●) mg/kg reserpine, and hearts were removed for NA analysis at various times, as shown. Each column or point represents a mean of 4 to 8 experiments. Vertical lines above columns and points show s.e. mean.

t test (two-tailed) was used for statistical analysis of the difference.

Reserpine treatment

Reserpine (Serpasil, Ciba Pharmaceutical Company) was administered intraperitoneally to rats in different single doses. In order to inject small quantities, a stock solution of reserpine was diluted with 0.9% w/v NaCl solution (saline) before injection. Tissues were removed for study 24 h later.

Results

Depletion and recovery of noradrenaline content of the heart after reserpine administration

As little as 0.005 mg/kg reserpine, injected 24 h previously, produced about 25% reduction in endo-

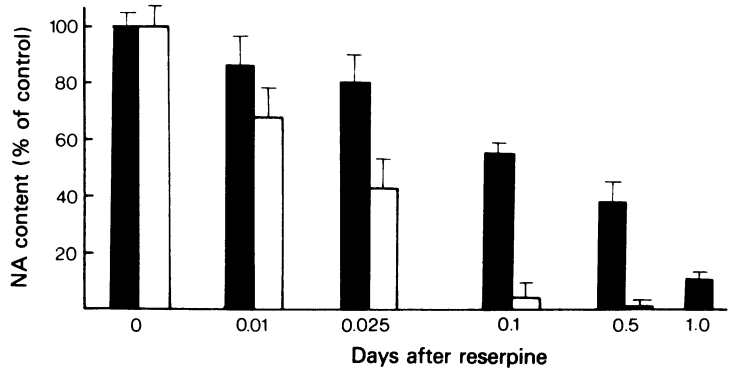

Figure 2 Effect of various doses of reserpine on the depletion of noradrenaline content of the superior cervical ganglion and salivary gland. Rats were injected with various doses of reserpine as shown, and their superior cervical ganglia (solid columns) and salivary glands (open columns) were removed after 24 h for NA analysis. (Control NA values for superior cervical ganglia and salivary glands were 20.38 ± 1.02 and 2.13 ± 0.19 μg/g, respectively, $n = 14$.) Each column represents a mean of 6 experiments. Vertical lines above each column represent s.e. mean.

genous NA content of the rat heart (Figure 1a). The reduction was significantly different ($P < 0.025$) when compared with the control. A further increase in the dose of reserpine to 0.01 mg/kg caused over 50% depletion, and 0.10 mg/kg almost completely depleted cardiac NA stores.

Recovery of the NA content of the heart was investigated in three groups of rats injected with 0.01, 0.1 or 10.0 mg/kg doses of reserpine. The results of these experiments are shown in Figure 1b: 0.01 mg/kg reserpine caused about 60% and the other doses between 90% and almost complete loss of NA content within 24 h. The rates of recovery were practically identical in the hearts of three groups of rats at 4, 8 and 14 days after reserpine administration. Cardiac NA stores were below 50% 14 days after the administration of 0.1 or 10 mg/kg reserpine in both cases.

Depletion and recovery of noradrenaline content of the superior cervical ganglion and salivary gland after reserpine administration

In order to study NA depletion by reserpine of nerve terminals and cell bodies of the same sympathetic neurone, the right salivary gland and superior cervical ganglion were removed for NA analysis. Results of these experiments are shown in Figure 2: 0.01 mg/kg reserpine caused a significant (30%, $P < 0.05$) reduction of NA content of the salivary gland without affecting the NA content of the superior cervical ganglion. A further increase in the dose to 0.025 mg/kg led to almost a 50% reduction in the salivary gland NA content. Although this dose of reserpine appeared to reduce the NA content of the superior cervical ganglion by about 20%, the difference was not statistically significant ($P < 0.1$). At a dose of 0.1 mg/kg reserpine, which caused over 90% reduction of NA content of the salivary gland, did produce about 50% depletion from the superior cervical ganglion;

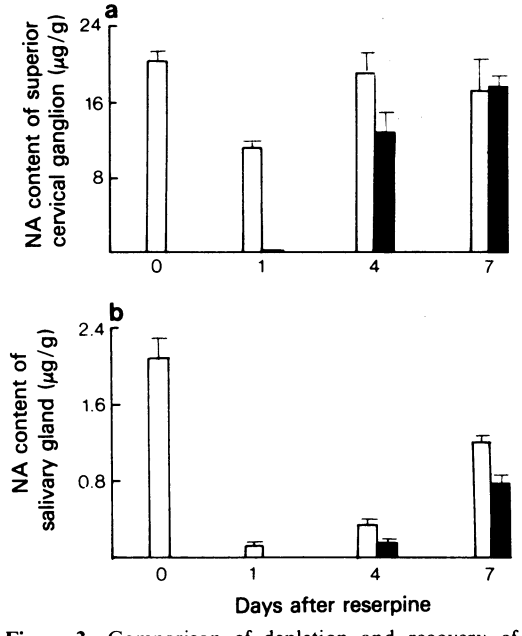

Figure 3 Comparison of depletion and recovery of noradrenaline (NA) content of superior cervical ganglion and salivary gland after reserpine. Rats were injected with 0.1 (open columns) or 10.0 (solid columns) mg/kg reserpine. Superior cervical ganglia (a) and salivary glands (b) were removed at various times, as shown, for NA analysis. Each column represents a mean of 6 to 14 experiments. Vertical lines above each column represent s.e. mean.

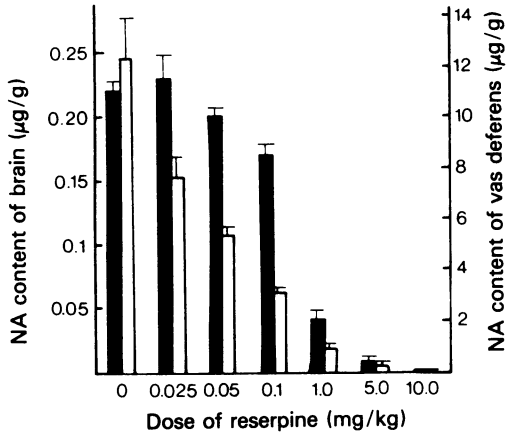

Figure 4 Effect of various doses of reserpine on noradrenaline (NA) content of the brain and vas deferens. Rats were injected with different doses of reserpine, as shown, and their whole brains (solid columns) and vasa deferentia (open columns) were removed after 24 h for NA analysis. Each column represents a mean of 5 experiments. Vertical lines above each column represent s.e. mean.

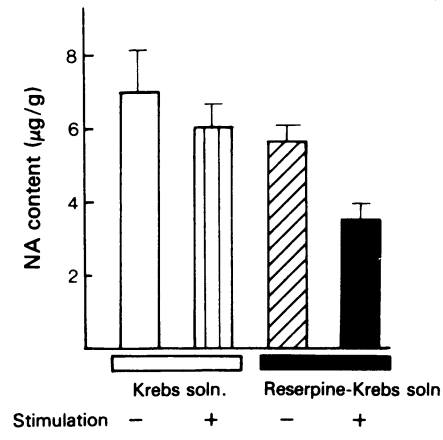

Figure 5 Effect of reserpine and transmural stimulation on noradrenaline (NA) content of the isolated left atrium of the guinea-pig. Left atrium was split in half. One half was transmurally stimulated for 30 min. The other half was kept in Krebs solution for 30 min. In another series of experiments, both halves of the atrium were kept in 5 μg/ml reserpine-Krebs solution for 30 min, but one half was transmurally stimulated, as stated above, for 30 min. Each column represents a mean of 6 experiments. Vertical lines above each column represents s.e. mean.

(10 mg/kg) was required to obtain over 90% depletion of the superior cervical ganglion.

Additional experiments were carried out to study the recovery of NA stores of the superior cervical ganglion and salivary gland following 0.1 and 10 mg/kg reserpine. The results of these experiments are shown in Figure 3. The ganglionic NA content was reduced by about 50% 24 h after 0.1 mg/kg, and was restored to almost normal levels in about 4 days. Even after complete depletion with a high dose of reserpine, the NA content was restored to about 50% in 4 days, and almost complete recovery was seen after 7 days (Figure 3a). On the other hand, the NA content of the salivary gland was reduced by about 90% after either dose of reserpine and remained at this low level even 4 days after injection. On the 7th day the NA content of the salivary gland was restored to about 40 to 60%.

Depletion of noradrenaline content of the vas deferens and brain after reserpine administration

Figure 4 shows the effect of various concentrations of reserpine on NA contents of the vas deferens and whole brain of the rat. The NA content of the brain was lowered significantly (25%, $P < 0.05$) only when the dose of reserpine was raised to 0.1 mg/kg. A further increase in reserpine dose to 1 mg/kg led to 80% depletion, and in order to obtain amost complete NA depletion a 10 mg/kg dose had to be given. In the case of the vas deferens, a substantial reduction in its NA content was found after administration of 0.025 mg/kg reserpine. A progressively greater degree of depletion was obtained as the dose of reserpine was raised.

Effect of transmural stimulation and reserpine on noradrenaline content of the isolated left atrium of the guinea-pig

The results of these experiments are shown in Figure 5. The mean NA content of 8 atria averaged 3.65 μg/g. Thirty min after transmural stimulation of the atria there was no significant change in NA content of the tissue. *In vitro* exposure to reserpine (5 μg/ml) for 30 min appeared to cause a modest but statistically insignificant ($P < 0.5$) drop in NA content of the atrium. However, reserpine together with transmural stimulation for 30 min led to 51% reduction in NA content as compared to that of control tissue kept in Krebs solution. The degree of NA depletion was 38% ($P < 0.05$) when compared with the NA content of the atrium kept in reserpine-Krebs solution for 30 min.

Effect of tetraethylammonium and reserpine and transmural stimulation on noradrenaline content of the isolated vas deferens of the rat

TEA, which facilitates NA overflow upon electrical stimulation of the vas deferens by almost 20 fold at 5

Hz, lowers the NA content of the transmurally stimulated vas deferens by about 70% in 2 h (Wakade, 1978b). Transmural stimulation alone for 2 h does not lower the NA content (Wakade, 1978b). *In vitro* treatment of the vas deferens with reserpine and transmural stimulation for 2 h results in about 70% reduction in tissue NA content (Wakade, 1978a). Therefore, it was decided to see if TEA plus reserpine could further reduce NA stores of the isolated vas deferens during transmural stimulation. The results of these experiments are shown in Figure 6. The reduction in the NA of the vas deferens kept in TEA and reserpine-Krebs solution for 2 h was 24% ($P < 0.05$). However, in the presence of transmural stimulation, TEA plus reserpine reduced the NA levels of the tissue in 2 h to 0.95 ± 0.07 μg/g. This depletion (90%) was greater than that found with either TEA or reserpine in the presence of stimulation (see above).

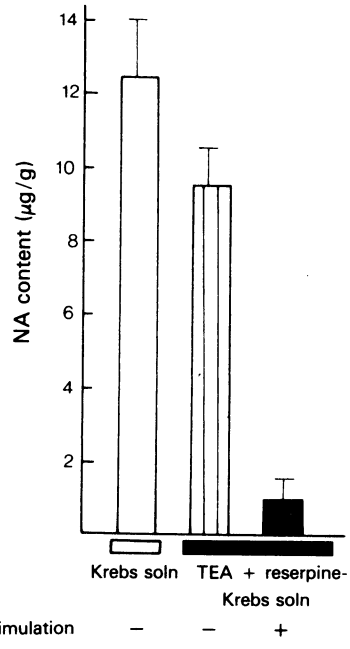

Figure 6 Effect of tetraethylammonium (TEA) and reserpine and transmural stimulation on noradrenaline (NA) content of the rat vas deferens. NA content of vas deferens kept in Krebs solution for 2 h is shown by open column. In another series of experiments, right (striped column) and left (solid column) vasa deferentia were kept in 1.5 mg/ml TEA and 5 μg/ml reserpine-Krebs solution for 2 h, but the left vas deferens was transmurally stimulated (5 Hz, 30 s/min) for 2 h. Each column represents a mean of 5 experiments. Vertical lines above each column represent s.e. mean.

Discussion

Among various sympathetic neuroeffector organs of the rat, the heart appears to be the organ most sensitive to the depleting action of reserpine. A single dose of 5 to 10 μg/kg produced about 20 to 60% depletion of cardiac NA stores in 24 h. In order to obtain a similar degree of NA depletion from the sympathetic nerves of other organs, such as the salivary gland and vas deferens, the reserpine dose had to be increased to 10–20 and 20–50 μg/g, respectively. The reason for the differences in NA depletion in these organs after reserpine is not known. The blood flow, and thereby the availability of reserpine to the organ, may play some role in causing the different degrees of NA depletion in the various organs. However, studies of Norn & Shore (1971) show that 24 h after a dose of reserpine, its concentration in various organs, such as spleen and adrenal gland, was actually greater than that in the heart. Therefore, better delivery of reserpine to the heart cannot be the main reason for the marked depletion seen in this organ. On the other hand, it has been proposed by a number of workers that nerve impulse activity is essential for the reserpine action (rabbit adrenal gland, Brodie, Olen, Kuntzman & Shore, 1957; rat adrenal gland, Dixon, Garcia & Kirpekar, 1976; cat salivary gland, Hertting, Potter & Axelrod, 1962; rabbit intestine, Kärki, Paasonen & Vanhakartano, 1959; anococcygeus muscle, Gillespie & McGrath, 1974).

In an attempt to obtain more direct evidence in favour of a neurogenic contribution to NA loss from sympathetic nerve terminals by reserpine, it was shown in the present study that reserpine lowers the NA content of the guinea-pig atrium more effectively when intramural nerve terminals of the tissue are excited. Previously, it was shown that the rat vas deferens exposed to reserpine *in vitro* for 2 h produced a slight reduction in the NA content; however, stimulation of nerve terminals in the presence of reserpine-Krebs solution led to 70% loss in NA content (Wakade, 1978b). The number of storage vesicles participating in the release of NA must increase during stimulation as compared to that in the nonstimulation period. If one believes that these vesicles once again synthesize NA and are involved in the storage and release process, then it is obvious that after reserpine these vesicles cannot synthesize NA due to the inhibition of the amine pump by reserpine (Carlsson, Hillarp & Waldeck, 1963). The net effect would be a greater reduction in NA content by reserpine during stimulation than at rest.

In support of such a concept, it was found that TEA increased the degree of NA depletion produced by reserpine plus nerve stimulation. In the presence of TEA and nerve stimulation, a still greater number of vesicles would participate in release, and subsequently become nonfunctional for new synthesis of NA in the

presence of reserpine. From these observations it seems that a large part of the action of reserpine is dependent upon the activity of the sympathetic nerves. If this is true, then it is tempting to speculate that the sympathetic nerves of the heart are more active than those innervating the salivary gland or the vas deferens. The differences in nerve activity may play a role in the variations in NA depletion in different organs after *in vivo* administration of reserpine. The difference in NA depletion in various organs of the guinea-pig treated with α-methyl-tyrosine were attributed to variations in the turnover rates of NA synthesis (Spector, Sjoerdsma & Udenfriend, 1965).

Very high concentrations of reserpine were needed to lower the NA content of the brain and superior cervical ganglion as compared to the heart. The brain and superior ganglion contain not only nerve terminals, but a large number of cell bodies as well. As discussed above, if the depleting action of reserpine is dependent on the nerve activity, one can infer that neurogenic activity is more in the terminal region than in the cell body of the sympathetic neurone.

The differential rates of recovery of NA in the cell body vs. the nerve terminals after reserpine treatment (Carlsson, Falck & Hillarp, 1962; Dahlström, Fuxe & Hillarp, 1965) may be related to a given population of newly synthesized storage vesicles in different regions of the sympathetic neurone. Storage vesicles are manufactured in the cell body and subsequently transported to the terminal portions of the neurone (Dahlström & Haggendal, 1966). If the rate of NA synthesis is similar in all regions of the neurone, then it is likely that after reserpine administration a greater population of newly formed vesicles in the cell body would acquire more NA than those in the nerve terminals. It is also possible that the 'old vesicles' affected by reserpine may contribute to the restoration of NA content in the cell body as well as in the terminals. The present results provide no direct evidence for either of these possibilities.

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