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# STUDIES ON THE RELATIONSHIP BETWEEN ADRENERGIC NERVE FUNCTION AND GRANULAR UPTAKE MECHANISMS

BY

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Treatment of animals with reserpine depletes the stores of noradrenaline in the tissues and also reduces the response of various organs to sympathetic nerve stimulation (Bertler, Carlsson & Rosengren, 1956; Holzbauer & Vogt, 1956; Muscholl & Vogt, 1958; Trendelenburg & Pfeffer, 1964). Andén, Magnusson & Waldeck (1964) have indicated that after treatment with reserpine there is no clear-cut relationship between the reduced tissue levels of noradrenaline and the interruption of sympathetic nerve transmission. This observation was recently confirmed and extended by Andén & Henning (1966) who showed that the noradrenaline content of the nictitating membrane of the cat was very low when the restoration of nerve function occurred. Lundborg (1963) has suggested that there is a better correlation between the return of the uptake capacity of adrenergic nerve granules and nerve function than there is between total tissue amine levels and functional aetivity. In the present investigation an attempt has been made to correlate the reappearance of physiological function after reserpine treatment with the return of uptake ability of catecholamine-containing granules isolated from sympathetically innervated tissue.

## METHODS

Mice, in groups of six, were given [$^3$H]-noradrenaline ([$^3$H]-NA), 1 $\mu$g/kg (6 c/mM) or [$^3$H]-$\alpha$-methylnoradrenaline ([$^3$H]-$\alpha$-MeNA), 100 $\mu$g/kg (30 mc/mM) intravenously. In some experiments reserpine (10 mg/kg) was given intraperitoneally at various intervals before the injection of the labelled amine. The animals were killed 15 min after the administration of [$^3$H]-$\alpha$-MeNA and 30 min after receiving $^3$H-NA. Hearts were removed and homogenized in an ice bath using a plastic pestle in a glass homogenizer.

The coarse particles were removed by centrifuging the homogenate at 4° C at 2000 *g* for 10 min. The resulting supernatant was then centrifuged at 100,000 *g* for 60 min in a Spinco Model L ultracentrifuge at 4° C, which gave the particulate fraction and the high speed supernatant used in this work. After protein precipitation, extracts of the various fractions were passed through cation exchange resin columns. The catecholamine was eluted with hydrochloric acid and the tritium content of the eluate was determined by liquid scintillation counting. Details of the above procedures have been described previously (Lundborg & Stitzel, 1967). Body temperatures of the mice were monitored using a Yellow Springs Instrument Co. Tele-Thermometer. Mice used for uptake studies were kept at 31° C. In studies of the effect of reserpine on the body temperatures the mice were kept at either 31° C or 23° C.

## RESULTS

### *Effect of reserpine pretreatment on the uptake of [$^3H$]-NA and [$^3H$]-α-MeNA into the particulate fraction of the mouse heart*

When [$^3$H]-NA was given to animals pretreated with reserpine, there was an almost complete blockade of uptake of the tritiated amine into the particulate fraction which lasted up to 12 hr after the injection of reserpine (Fig. 1). During the next 12 hr there was a small, but gradual, increase in the uptake of [$^3$H]-NA into the granules, while between 24 and 48 hr after administration of reserpine a marked increase in amine uptake was observed. Thereafter, a continual recovery of granular uptake function occurred until at 48 and 96 hr the uptake of the granules was about 35 and 80% of normal, respectively.

The uptake of [$^3$H]-α-MeNA by the granules was reduced to about 30% of control within 0.5 hr after a single injection of reserpine (Fig. 1). This degree of inhibition

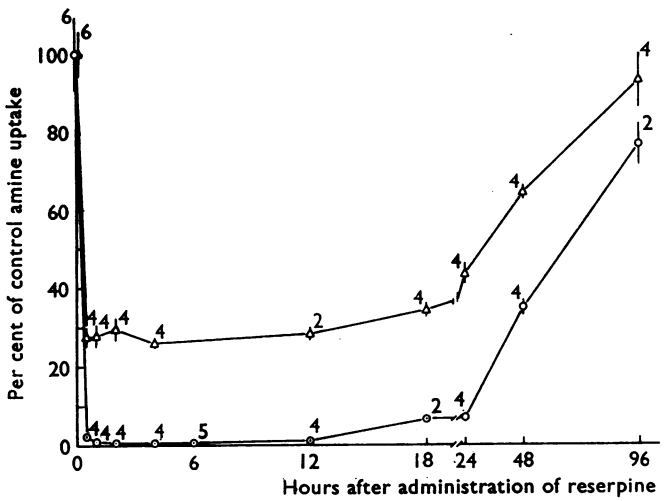


Fig. 1. Effect of reserpine on the uptake of [$^3$H]-NA (○——○) and [$^3$H]-α-MeNA (△——△) into particulate fraction of the mouse heart. Vertical lines indicate standard errors of the mean and the figures the numbers of experiments.

remained relatively constant for up to 12 hr and then gradually diminished during the next 12 hr period. Between 24 and 48 hr after reserpine administration, there was a marked increase in the ability of the granules to take up [$^3$H]-α-MeNA until at 48 hr the uptake was approximately 70% of normal.

### *Effect of reserpine pretreatment on the distribution of [$^3H$]-NA and [$^3H$]-α-MeNA between subcellular fractions of the mouse heart*

When the amount of labelled amine in the particulate fraction was expressed as a percentage of that found in the particulate plus supernatant fraction ($P/(P+S) \times 100$), [$^3$H]-NA was found to be present in a slightly greater relative concentration (45%) than

was [$^3$H]-$\alpha$-MeNA (42%) (Fig. 2). Mice pretreated with reserpine, however, showed a greatly reduced distribution ratio. This effect was particularly marked during the first 12 hr after the administration of reserpine and then gradually disappeared. The distribution of [$^3$H]-NA had returned essentially to normal 18–48 hr after the injection of reserpine while that of [$^3$H]-$\alpha$-MeNA did not return to normal until 96 hr.

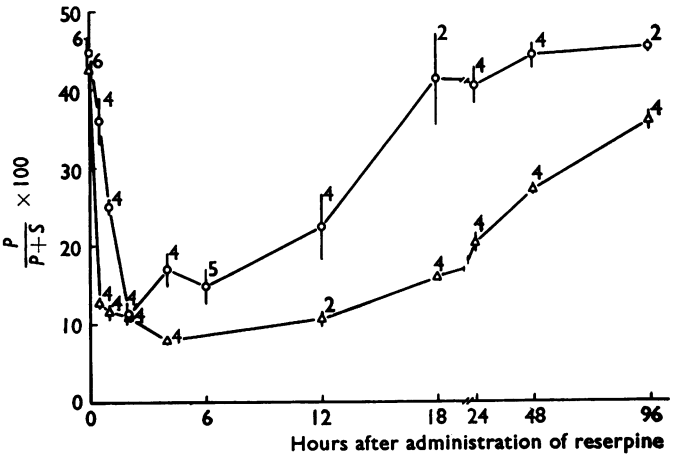


Fig. 2. Effect of reserpine on the subcellular distribution of [$^3$H]-NA (○——○) and [$^3$H]-$\alpha$-MeNA (△——△) in the mouse heart. Vertical lines indicate standard errors of the mean and the figures the number of experiments. *P*, Amount of labelled amine in particulate fraction; *S*, amount of labelled amine in supernatant fraction.

*Effect of reserpine on body temperature of mice maintained at 23° C*

After a single dose of reserpine, mice kept at an ambient temperature of 23° C showed an immediate fall in body temperature (Fig. 3). Their temperature dropped from a pre-injection value of 38.3° C to about 25° C within 6 hr. The temperature of the mice

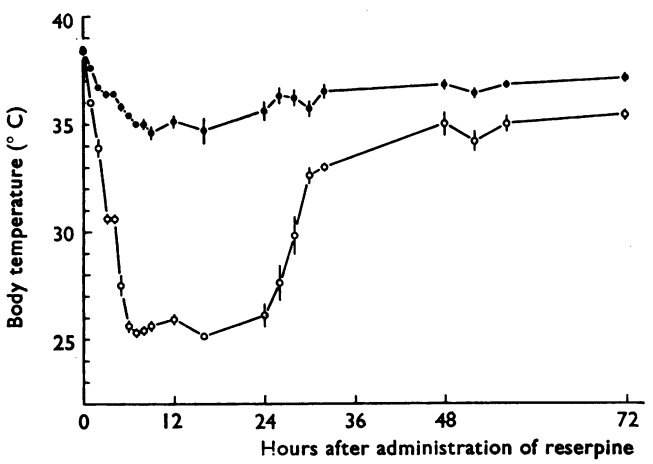


Fig. 3. Effect of a single dose of reserpine on the body temperature of mice maintained at either 23° (○——○) or 31° C (——●). Vertical lines are standard errors of the mean.

remained at this level for the next 18 hr and then showed a marked increase during the period of 24–48 hr after administration of reserpine. Even at the end of 72 hr, however, the mice could not maintain their temperature at completely normal levels.

*Effect of reserpine on body temperature of mice maintained at 31° C*

If mice were kept in an environment of 31° C after the intraperitoneal injection of reserpine 10 mg/kg, they were able to maintain their body temperature much better than animals kept at 23° C (Fig. 3). The reduction in temperature was much smaller for the former (38.5°–34.6°) than for the latter (38.3°–25.1°) animals. If the mice kept at 31° C were exposed to a temperature of 23° C for 1 hr 4–24 hr after the reserpine injection, however, their ability to maintain body temperature was still markedly impaired. The exposure for 1 hr produced a drop in temperature ranging from 4.0° to 5.8° C during the first 24 hr after the reserpine injection (Table 1). By 31 hr, however, a decrease in ambient temperature resulted in a fall in body temperature of only 0.8°.

TABLE 1

EFFECT OF A SINGLE DOSE OF RESERPINE ON THE ABILITY OF MICE TOMA INTAIN BODY TEMPERATURE

The animals were kept at an ambient temperature of 31° C and were given reserpine (10 mg/kg I.P.). After various intervals they were subjected to a temperature of 23° C for 1 hr. Each group consisted of six animals.

| Hours after reserpine at 31° C | Body temperature | | |
|---|---|---|---|
| | Before transfer to 23° C | After 1 hr at 23° C | Mean drop |
| 2 | 37·3±0·08 | 34·5±0·34 | 2·8±0·4 |
| 3 | 36·9±0·15 | 34·4±0·30 | 2·5±0·2 |
| 4 | 36·5±0·15 | 32·5±0·59 | 4·0±0·6 |
| 8 | 35·4±0·20 | 29·6±0·28 | 5·8±0·3 |
| 18 | 34·8±0·34 | 30·8±0·47 | 4·0±0·4 |
| 24 | 37·2±0·33 | 33·0±0·34 | 4·2±0·5 |
| 31 | 35·4±0·22 | 34·7±0·33 | 0·8±0·3 |

## DISCUSSION

In the present work an attempt has been made to correlate adrenergic nerve function and the incorporation of amines into particles containing catecholamines.

We have studied the ability of an animal to maintain its body temperature as an index of the pharmacological effects of reserpine. A single injection of a large dose of reserpine causes a fall in body temperature in mice. Body temperature reaches a minimum about 6 hr after administration of reserpine and remains at this level for about 18 hr. The ability of adrenergic granules to retain [$^3$H]-NA and [$^3$H]-$\alpha$-MeNA is also blocked by reserpine and the time-course of temperature depression and uptake inhibition correlate quite well. Granular uptake mechanisms are almost completely inhibited within the first 30 min and body temperature begins to fall within 1 hr after drug treatment. The relatively slow decline in temperature during the first 6 hr probably represents the time necessary to deplete the residual noradrenaline stores present in the nerves.

About 24 hr after the reserpine injection, body temperature begins to rise and it is at this time that the particles containing noradrenaline show the first marked return of uptake ability. It should be noted that endogenous amine levels are still very low at this

time (Carlsson, Rosengren, Bertler & Nilsson, 1957). The almost simultaneous recovery of function and uptake ability despite a severe depletion of the noradrenaline stores suggests that adrenergic transmission is more closely dependent on the ability of the storage granules to incorporate noradrenaline than on amine levels. The very low amine levels at functional recovery imply that the amine pool necessary for nerve function is small.

If animals treated with reserpine are kept in an environment of 31° C they are much better able to maintain their body temperature. This is probably the result of a diminished heat loss rather than any modification of noradrenaline nerve function brought about by the elevated ambient temperature. This is supported by the finding that these animals show a rapid fall in body temperature if they are placed at 23° C for 1 hr. This effect is seen during the first 24 hr after administration of reserpine. After that time, exposure to 23° C produces only a minimal fall in temperature. The ability to maintain body temperature after acute exposure to 23° C coincides well with the previously noted return of uptake ability of the nerve granules. Hoffman (1958) has also shown that animals given reserpine show a minimal temperature 24 hr after an injection of reserpine, but begin to recover shortly after that time.

Other symptoms secondary to reserpine treatment, such as ptosis, miosis and diarrhoea, may also be related to the blockade of the uptake of amines into storage particles. Stjärne (1964) and Iversen, Glowinski & Axelrod (1965) have found that there is a better correlation between " clinical " recovery and the re-establishment of uptake than between recovery and repletion of the emptied noradrenaline stores.

The subcellular distribution of [$^3$H]-NA had returned essentially to normal 48 hr after the injection of reserpine while that for [$^3$H]-$\alpha$-MeNA did not return to normal until 96 hr (Fig. 2). This difference between the two amines is based on reliable data and calls for comment.

During the first few hours after administration of reserpine, almost all [$^3$H]-NA taken up by the nerve cell is probably rapidly destroyed by the intracellular monoamine oxidase. The first signs of recovery in the particulate fraction, noticed after 18–24 hr, would indicate the first recovery of a small granular fraction. This fraction is apparently not large enough to take care of all [$^3$H]-NA taken up by the nerve cell membrane pump, but is evidently enough to prevent a certain amount of the amine from being destroyed. This destruction of extragranular [$^3$H]-NA by monoamine oxidase probably explains why an approximately normal distribution ratio is already obtained 18–24 hr after reserpine.

[$^3$H]-$\alpha$-MeNA is resistant to monoamine oxidase, and thus there is an accumulation of the amine in the sympathetic nerve even if granular uptake mechanisms are impaired. Thus the $P/(P+S)$ ratio does not return to control values until the uptake function is fully recovered.

It can be concluded that a certain amount of granular function has already been recovered 18–24 hr after reserpine treatment. The time needed for complete recovery of the granular uptake function, however, seems to be at least 96 hr.

The studies of Dahlström (1966, 1967) have shown that fresh, uninhibited granules have begun to reach the nerve terminals within 24 hr after administration of reserpine. The recovery of granular uptake after reserpine treatment may be explained by the

formation of new storage granules within the sympathetic nerve and their subsequent transport down the axons to the nerve terminals (Dahlström & Häggendal, 1966). The possibility of a reversal of the inhibition induced by reserpine cannot, however, be ruled out.

The present study suggests that the return of sympathetic function is related to granular uptake mechanisms, but cannot distinguish between the arrival of new granules and reversal of granular inhibition as the underlying cause.

## SUMMARY

1. The body temperature of mice and the uptake *in vivo* of [$^3$H]-NA and [$^3$H]-$\alpha$-MeNA into subcellular fractions of the mouse heart after a large dose of reserpine was studied.

2. A single injection of a large dose of reserpine causes a pronounced fall in body temperature in mice.

3. The ability of adrenergic granules to take up [$^3$H]-NA and [$^3$H]-$\alpha$-MeNA is also blocked by reserpine, and the time course of temperature depression and uptake inhibition correlates quite well.

4. The almost simultaneous recovery of nerve function and uptake ability despite severe depletion of the noradrenaline stores suggests that adrenergic transmission is more closely related to granular uptake mechanisms than to total amine content.

5. The possible causes for the return of granular uptake 24–48 hr after reserpine treatment are discussed.

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