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# The uptake and retention of [$^3$H]noradrenaline in rat sciatic nerves after ligation

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The uptake and retention of [$^3$H]noradrenaline ($^3$H-NA) was examined in sciatic nerves of albino rats. In the 1 cm part of nerve proximal to a 12 hr ligation the uptake and retention of exogenous noradrenaline was about 4 times the uptake in 1 cm of normal unligated nerves. Treatment with reserpine 10 hr before killing caused a marked decrease in the estimated amount of $^3$H-NA, while injection of nialamide 15 min before $^3$H-NA administration in ligated, reserpine-treated animals caused a somewhat larger uptake and retention of $^3$H-NA in the nerve part above the ligation. Protriptyline, a blocker of the "membrane pump", was approximately 3 times less effective in 12 hr-ligated nerves than in unligated nerves, indicating a reduced efficiency of the "membrane pump" in the distended axons above a ligation.

THE accumulation of noradrenaline proximal to a ligation in peripheral adrenergic nerves has been noted previously (Dahlström & Fuxe, 1964; Dahlström, 1965; Dahlström & Häggendal, 1966; 1967; Kapeller & Mayor, 1966). Reserpine, which is known to empty the monoamine stores in central and peripheral tissues by blocking the storage mechanism of the amine storage granules (*cf.* Carlsson, Hillarp & Waldeck, 1963; Carlsson, 1965; Dahlström, Fuxe & Hillarp, 1965; Malmfors, 1965), depletes the noradrenaline accumulated in sciatic nerves above a ligation (Dahlström, 1965; 1967a). If the monoamine oxidase inhibitor nialamide is given before reserpine to nerve ligated rats the noradrenaline fluorescence is unchanged (Dahlström, 1967a). For these reasons it has been assumed that the accumulation of noradrenaline occurring proximal to a ligation in adrenergic axons is due to a piling up of noradrenaline storage granules transported proximo-distally in the axons (Dahlström, 1966; 1967a; b; Dahlström & Häggendal, 1966; 1967).

The uptake and retention of [$^3$H]noradrenaline ($^3$H-NA) in adrenergic nerves of normal animals has been shown to be dependent on the efficiency of the amine transport mechanism at the level of the cell membrane (the so called "membrane pump") (Hillarp & Malmfors, 1964; Carlsson & Waldeck, 1965a; Lindmar & Muscholl, 1964). This transport mechanism can be blocked by protriptyline (Carlsson & Waldeck, 1965b; Malmfors, 1965). The uptake and retention of $^3$H-NA is also dependent on the presence of functioning amine storage granules (cf. Lundborg & Stitzel, 1967). Therefore it was thought of interest to examine the uptake of $^3$H-NA in the sciatic nerve of the rat under different conditions. In the present study the influence of ligation, reserpine, nialamide and protriptyline was investigated.

## Experimental

### MATERIAL AND METHODS

Male albino rats of the Sprague–Dawley strain (180–200 g) were given a single intravenous injection of $^3$H-NA (specific activity 10 c/mmole)

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5 μg/kg 30 min before death. The animals with bilateral ligation of the sciatic nerve were operated under light ether anaesthesia 12 hr before death. Reserpine (Serpasil ampoules 2·5 mg/ml, CIBA) was given intraperitoneally in one single dose of 10 mg/kg 10 hr before killing. Nialamide was administered intraperitoneally to reserpine-pretreated animals with ligations either 2 hr (10 mg/kg) or 15 min before $^3$H-NA injection (100 mg/kg). Protriptyline was given intravenously (10 mg/kg) to normal rats or to rats with 12 hr bilateral ligations 10 min before the injection of $^3$H-NA. Before the intravenous administration of $^3$H-NA, the rats were anaesthetized with pentobarbitone (35 mg/kg).

The animals were killed by decapitation and the 1 cm part of the nerve just above the ligation was dissected. Nerves from 2 rats were always pooled, so too were the nerves from unligated rats where 3 cm of nerve on each side was dissected. The nerve parts were put into 10 ml of ice-cooled perchloric acid (0·4N), homogenized and centrifuged. Noradrenaline was separated from the supernatant by chromatography on columns of a strong cation-exchange resin (Dowex 50W X4), and the radioactive noradrenaline was then estimated by liquid scintillation counting essentially as described by Carlsson & Waldeck (1963).

## Results

The uptake and retention of $^3$H-NA in 1 cm of normal unligated rat sciatic nerve was found to be 2·6 pg as a mean. Twelve hr after ligation, the amount had increased to 9·3 pg in the 1 cm just proximal to the ligation. Reserpine treatment 10 hr before death reduced the amount of recovered $^3$H-NA in ligated nerves to about 0·7 pg/cm. In reserpine-treated animals, nialamide caused an increase in the amount of $^3$H-NA, particularly in the group given a high dose 15 min before the $^3$H-NA administration. In normal rats, given protriptyline 10 min before $^3$H-NA, the amount of $^3$H-NA recovered was reduced to about a quarter. In ligated rats, however, the effect of protriptyline was much less pronounced, the amount of $^3$H-NA recovered was reduced only by a factor of 1·4.

## Discussion

In earlier reports, the proximo-distal transport of noradrenaline in the adrenergic axons has been described. In the rat the rate of this transport has been calculated to be about 5 mm/hr (Dahlström & Häggendal, 1966). There are several reasons to believe that the measured noradrenaline content in normal and ligated nerves is stored within granules and that these granules are formed in the nerve cell body and transported down to the nerve terminals via some kind of active transport mechanism in the axons (see Discussions in Dahlström, 1965; 1966; Dahlström & Häggendal, 1966). One piece of evidence for this view is the disappearance of noradrenaline fluorescence on reserpine treatment; and that this disappearance can be inhibited by pretreatment with nialamide (Dahlström, 1966; 1967). Such behaviour of the noradrenaline is consistent with the idea that the amine is stored within granules (Carlsson, 1965; Malmfors, 1965).

In the present experiments it was found that 12 hr after ligation the uptake and retention of labelled noradrenaline was much larger compared to that in unligated nerves. Reserpine treatment caused a great decrease in the amount of $^3$H-NA recovered, probably due to the granule-blocking effect of this drug. Inhibition of monoamine oxidase in reserpine-pretreated animals caused some increase in the $^3$H-NA recovered particularly when a relatively large dose of nialamide was given at a short interval before $^3$H-NA administration. There exists a very subtle balance between enhancing and antagonizing effects of monoamine oxidase inhibitors on the uptake and retention of $^3$H-NA in reserpine-treated animals (Carlsson & Waldeck, 1967). In view of this it is not surprising that the time and dosage schedules for nialamide are critical (compare group E and F in Fig. 1). As seen from the Results, protriptyline (known

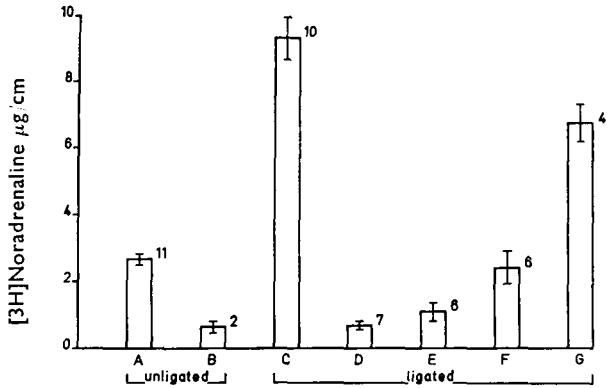


FIG. 1. Effect of drugs on the uptake and retention of [$^3$H]noradrenaline ($^3$H-NA) in the rat sciatic nerve. Rats, normal (unligated) and rats whose sciatic nerves had been ligated 12 hr beforehand (see text) received $^3$H-NA 1 µg/kg i.v. 30 min before being killed. The amount of $^3$H-NA/cm sciatic nerve (in ligated nerves the 1 cm just above lig.) was estimated. A and C, no drug, B and G, protriptyline 10 mg/kg i.v. 10 min before $^3$H-NA. D, reserpine 10 mg/kg i.p. 10 hr before $^3$H-NA. E, reserpine 10 mg/kg i.p. 10 hr and nialamide 10 mg/kg i.p. 2 hr before $^3$H-NA. F, reserpine 10 mg/kg i.p. 10 hr and nialamide 100 mg/kg i.p. 15 min before $^3$H-NA. Also shown are the mean ± s.e. Figures above bars indicate the number of observations.

to block the uptake of catecholamines across the nerve membrane, see above) caused a marked decrease in the recovery of $^3$H-NA in unligated nerves, suggesting that the non-terminal adrenergic axons in the sciatic nerve have mainly the same properties as the adrenergic nerve terminals. In ligated nerves, however, protriptyline had little effect (see below).

In earlier experiments on endogenous noradrenaline content in ligated sciatic nerves, the ratio between 1 cm of unligated nerve and the proximal 1 cm just above a 12 hr-ligation was found to be about 1 : 7. The present experiments reveal a difference in uptake and retention of exogenous noradrenaline in normal and ligated nerves of about 1 : 3·6. This discrepancy may be due to, for instance, the following possibilities. The procedure of ligation may reduce the blood supply to this nerve part.

This is unlikely since the ligation is made by a short-lasting compression of the nerve without any sideways tearing, and since peripheral nerves are supplied with blood by a mesoneurium sending vessels to the nerve perpendicularly. Also, experiments with tandem ligations and tetrabenazine [a short-lasting blocker of granular storage mechanism (Carlsson, Hillarp & Waldeck, 1963; Carlsson & Lindqvist, 1966; Häggendal, 1968)] have shown that granules in a nerve part separated by two ligations behave normally in respect of storing and synthesizing noradrenaline (Dahlström, 1967), indicating an unimpaired blood supply to the nerves. A second possibility which has to be discussed, is that a large part of the endogenous noradrenaline that accumulated above a 12 hr ligation (Dahlström & Häggendal, 1966) might be situated extragranularly in the cytoplasm, and that the real intragranular part was demonstrated by the uptake of exogenous noradrenaline. This can in all probability be ruled out, since with the sensitive histochemical fluorescence method used in earlier work very little or no noradrenaline fluorescence could be observed after treatment with reserpine. A third possibility is that the ratio of membrane-surface to volume is greatly decreased in the axons above a 12 hr-ligation compared to normal axons and nerve terminals, as pointed out earlier (Dahlström, 1965). Exogenous noradrenaline would theoretically have more difficulty in penetrating to the centrally located granules in these enlarged, bulky axons than in normal thin nerves and terminals. Finally, the explanation which seems to be most reasonable is that the amine uptake mechanism at the level of the cell membrane, the so called "membrane pump" (Hillarp & Malmfors, 1964; Lindmar & Muscholl, 1964; Carlsson & Waldeck, 1965a) is impaired by the distention of the axonal membrane during the process of accumulation. This last mentioned alternative is strongly supported by the results obtained with protriptyline which is known to block the membrane pump. In animals ligated 12 hr beforehand, this drug was approximately three times less effective than in unligated animals.

The results in the present experiments thus support the view that above a ligation of sympathetic adrenergic nerves, noradrenaline storage granules formed in the nerve cell body accumulate in large numbers. They also suggest an impairment of the uptake mechanism across the nerve membrane, possibly as a result of mechanical distention of the membrane.

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