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EFFECT OF RESERPINE ON RELEASE OF [^{3}H]NORADRENALINE, [^{3}H]DOPAMINE AND [^{3}H]METARAMINOL FROM FIELD STIMULATED RAT IRIS

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Abstract—The field stimulation induced release of [^{3}H]noradrenaline (^{3}H-NA)*, [^{3}H]-dopamine (^{3}H-DA) and [^{3}H]metaraminol (^{3}H-MA) from the isolated rat iris was investigated. Irides of untreated rats or rats pretreated with reserpine and/or the monoamine oxidase inhibitor nialamide were incubated with ^{3}H-NA, ^{3}H-DA or ^{3}H-MA. The irides were then superfused with physiological buffer and stimulated by an electrical field. In irides from untreated rats where ^{3}H-NA is taken up into the nerves and incorporated into the amine storage granules, field stimulation substantially enhanced the overflow of tritium. The greatest part of the tritium efflux during stimulation was recovered as ^{3}H-NA. Pretreatment with nialamide slightly decreased the total tritium overflow but increased the overflow of ^{3}H-NA. After reserpine (4 hr) and nialamide (1 hr), ^{3}H-NA taken up into the nerves is mainly located extragranularly in the axoplasm. In this case the stimulus induced overflow was almost completely abolished. This confirms the earlier finding that extragranular ^{3}H-NA cannot be released by electrical stimulation. ^{3}H-DA can after reserpine and nialamide partly be taken up into the amine storage granules by a reserpine-resistant mechanism and was to a certain extent released together with ^{3}H-NA newly formed from ^{3}H-DA. After reserpine ^{3}H-MA can be incorporated into the amine storage granules by a reserpine-resistant mechanism. The ^{3}H-MA, taken up after reserpine, was released to the same extent as ^{3}H-MA taken up in irides of untreated rats. It is concluded that granular storage of the transmitter is a prerequisite for depolarization induced release.

ADRENERGIC nerves can take up and store exogenous CA.[1–3] At least two different mechanisms seem to be responsible for this uptake and storage. One mechanism, the s.c. membrane pump, is localized to the nerve cell membrane and is an active, energy-requiring process which is of importance for the initial uptake of CA.[4–8] The other mechanism is localized to the amine storage granules and is dependent on Mg^{2+}-ATP.[9–12] Reserpine does not affect the membrane pump,[4–8,13] but effectively blocks the Mg^{2+}-ATP dependent uptake mechanism of the amine storage granules.[9–12]

In addition to the Mg^{2+}-ATP dependent uptake, also a reserpine-resistant uptake into the granules can be demonstrated.[14–16] The quantitative importance of this uptake is different for different amines.[14,17] DA can utilize both the Mg^{2+}-ATP dependent and the reserpine-resistant granular uptake mechanism, while NA preferentially uses the Mg^{2+}-ATP dependent and MA the reserpine-resistant mechanism.[14–16]

* Abbreviations used: CA = catecholamine(s), DA = dopamine, NA = noradrenaline, MA = metaraminol, NM = normetanephrine, MAO = monoamine oxidase, PCA = perchloric acid.

Exogenous ^{3}H-NA, taken up into the adrenergic nerves of untreated animals, can be released by nerve stimulation[18,19] or electrical stimulation *in vitro*.[20–25] Also MA taken up in adrenergic nerves[26,27] can be released by nerve stimulation.[28]

The aim of the present work was to study the significance of the subcellular localization of the transmitter for its release. The field stimulation induced release of ^{3}H-NA, ^{3}H-DA and ^{3}H-MA from isolated rat iris was investigated. Reserpine, which to different degrees affects the granular storage capacity for the different amines, was used to alter the subcellular distribution of the amines.

METHODS

Adult female albino rats (Sprague–Dawley, 180–200 g) were used for the experiments. Some of the animals were pretreated with reserpine (10 mg/kg i.p.) 4, 16, 24 or 48 hr and/or nialamide (100 mg/kg i.p.) 1 hr before sacrifice. If nothing else is stated, animals given reserpine were always given nialamide 1 hr before sacrifice. The animals were decapitated under ether anesthesia and the eyes were removed.

Incubation procedure. Isolated irides, with the ciliary bodies attached, were prepared and immediately immersed in a cool Krebs–Ringer bicarbonate buffer (in millimoles per liter: NaCl, 118; KCl, 4·8; $CaCl_2$, 1·3; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 25; ascorbic acid, 1·1; and glucose, 10). They were then incubated for 30 min at 37° with ^{3}H-NA, ^{3}H-DA or ^{3}H-MA (10^{-7} M or 10^{-6} M) in Krebs–Ringer medium, pH 7·4 equilibrated with 6·5% CO_2 in O_2.[8]

Stimulation procedure. After incubation the irides were transferred to small chambers where they were superfused with amine-free buffer (0·5 ml/min) at 37°. The chambers were made out of Plexiglass tubes (length 30 mm, inner diameter 4 mm) in which the iris could float freely between two nylon meshworks. At both ends of the chamber flat 2 mm wide platinum electrodes were placed. After 30 min superfusion the stimulation was started by generating an electrical field between the electrodes (biphasic pulses, 12 mA, 2 msec, 10 per sec) by a Grass S-4 stimulator.[21,22] Stimulation current was continuously monitored on a cathode ray oscilloscope. The irides were stimulated for 10 min and the superfusion continued for another 15 min. In some experiments the irides were after 30 min superfusion with drug-free buffer superfused with reserpine phosphate (10^{-7} M) for 30 min and then stimulated in the presence of reserpine phosphate.

Analytical procedures. In some experiments the superfusate was collected in 5 min fractions directly in the counting vials for determination of total radioactivity after the addition of 5 ml Insta-Gel® scintillation solution. In other experiments the superfusate was collected in 10 min fractions in 5 ml cool 0·8 N PCA for determination of ^{3}H-DA, ^{3}H-NA and their ^{3}H-metabolites. The irides were either dissolved in 0·5 ml Soluene® and radioactivity determined after the addition of 10 ml toluene scintillation solution, or homogenized in 3 ml cool 0·4 N PCA for analysis of ^{3}H-DA, ^{3}H-NA and their ^{3}H-metabolites.

After addition of 0·1 ml ascorbic acid (20 mg/ml), 0·2 ml EDTA Na_2 (100 mg/ml) and 10 μl carrier DA, NA and NM (1 mg/ml) the PCA extracts were neutralized to pH 6·5 by adding 5 N K_2CO_3 and were centrifuged 10,000 *g* for 10 min. The supernatants were chromatographed as follows.

Samples from experiments where irides had been incubated with ^{3}H-NA were chromatographed on Dowex 50W-X4 columns (diameter 4·0 mm, height at pH 1,

120 mm) according to Carlsson and Waldeck.[29] The effluent, containing neutral and acid catabolites of NA, was collected and after acidification to pH 2 shaken with 300 ml ether. After evaporation of the ether the radioactivity was taken up in 1 ml ethanol/0·01 N HCl and 5 ml toluene scintillation solution of which a 5 ml aliquot was counted.[30] The adsorbed amines were eluted with N HCl. The first 7 ml were discarded, the next 8 ml contained NA and the next 13 ml contained NM. Seven per cent NA contaminated the NM fraction and this was corrected for in the calculations. The recovery for NA and NM was about 80 per cent. No estimation of recovery for acid and neutral catabolites was made. It is likely, however, that the recovery of ^{3}H-deaminated catabolites was lower than that of ^{3}H-NA and ^{3}H-NM.[23] No corrections for recovery were made in the calculations.

Samples from experiments where irides had been incubated with ^{3}H-DA were chromatographed on Dowex 50W-X4 ion-exchange columns (diameter 4·0 mm, height at pH 1, 70 mm).[31] The adsorbed amines were eluted with N HCl and two fractions collected, the first 8 ml containing NA and the following 13 ml containing DA. Twenty per cent of the NA eluted contaminated the DA fraction and 4 per cent of the DA was found in the NA fraction. This was corrected for in the calculations. No clear separation of NM and DA is obtained using this technique. It has been shown, however, that negligible amounts of ^{3}H-NM are formed during incubation of isolated atria[31] or cerebral cortex slices[32] with ^{3}H-DA. The radioactivity found in the NA and the DA fractions thus almost exclusively represents ^{3}H-NA and ^{3}H-DA. No estimation was made of ^{3}H-deaminated catabolites. Recovery for DA and NA was about 80 per cent. No corrections for recovery were made in the calculations.

All eluates were freeze-dried in the counting vials and the amines taken up in 1 ml acid ethanol before addition of 15 ml toluene scintillation solution. Radioactivity was determined by liquid scintillation spectrometry in a Packard Model 3220. Quenching was determined by recounting representative samples after the addition of a standard amount of [^{3}H]toluene.

Calculations. The efflux of total tritium was plotted against time [see Fig. 1(a)]. *Stimulus induced efflux* (in dis./min) was calculated by subtracting the estimated spontaneous efflux [Fig. 1(a), dotted line, open circles] from the total efflux (continuous line, filled circles). Stimulus induced efflux was also expressed as per cent of the total tritium content in the irides at the onset of stimulation and was calculated from the formula:

$$\text{stimulus induced efflux} = \frac{\text{stimulus induced tritium efflux}}{\text{total tritium efflux from the onset of stimulation} + \text{tissue tritium content at the end of superfusion}} \times 100\%.$$

MATERIALS

The substances used were: *dl*-noradrenaline-7 ^{3}H-HCl, dopamine-7-^{3}H-HCl and *dl*-metaraminol-7-^{3}H-HCl (5–10 c/m-mole, New England Nuclear Corp., Boston, Mass.); *dl*-noradrenaline-HCl (Calbiochem.); dopamine-HCl (Sigma); normetanephrine-HCl (Sigma); reserpine (Serpasil®, Swedish Ciba); reserpine phosphate (Swedish Ciba); nialamide (Niamid®, Swedish Pfizer); Soluene®-100 and Insta-Gel® (Packard Instrument Company).

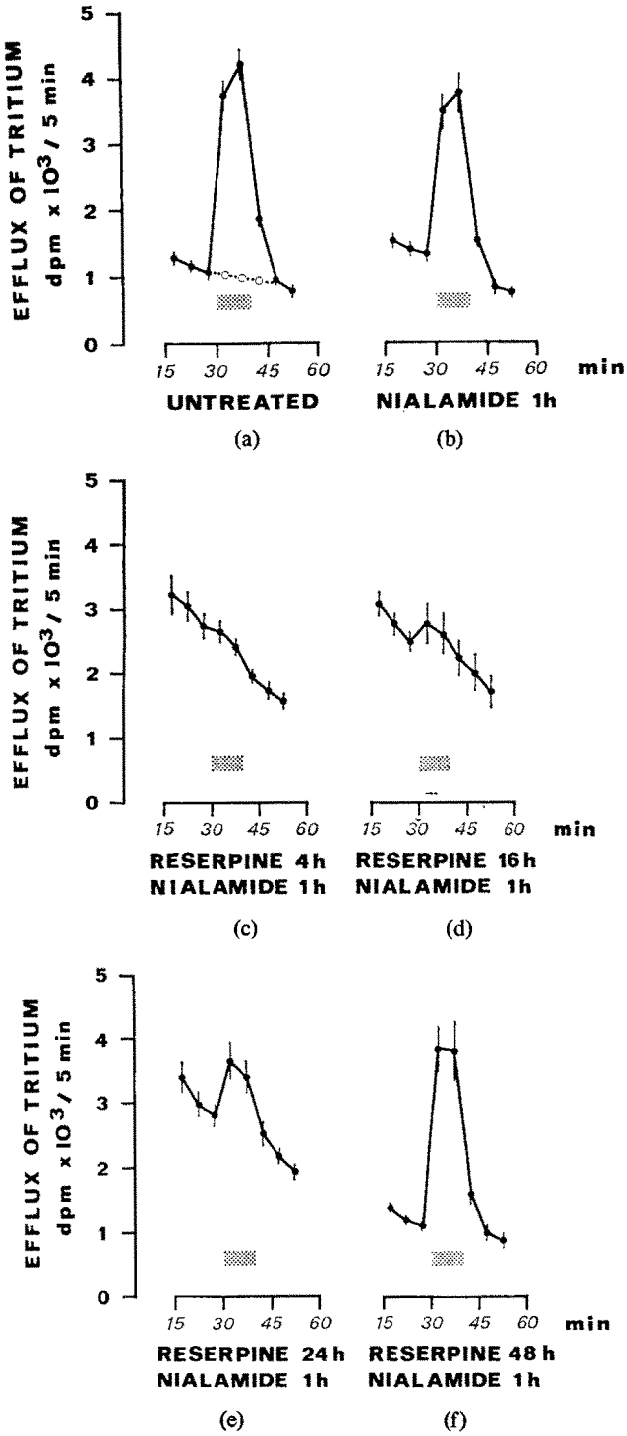

FIG. 1. Efflux of total tritium from isolated irides preincubated with ^{3}H-NA 10^{-7} M. Rats were pretreated as shown in the figures. The doses given were: reserpine 10 mg/kg i.p. and nialamide 100 mg/kg i.p.

●—● Efflux of total tritium.
○---○ Estimated spontaneous tritium efflux [Fig. 1(a) only].
▒ Stimulation.
Mean ± S.E.M. of 4–12 experiments.

RESULTS

Irides incubated with ^{3}H-NA. Isolated rat irides were incubated with ^{3}H-NA 10^{-7} M for 30 min and then superfused with fresh buffer. After 30 min superfusion they were stimulated by an electrical field for 10 min and then further superfused for 15 min (see Methods). Stimulation strongly enhanced the efflux of total tritium from untreated irides [Fig. 1(a)]. Between 80 and 90 per cent of the radioactivity recovered from the stimulation period and in the irides was unchanged ^{3}H-NA (Fig. 2). Of the total tritium content present in the tissue at the onset of stimulation 12 per cent was overflowing into the superfusate in response to stimulation (Table 1).

Nialamide pretreatment which allowed a higher accumulation of tritium in the tissue (Table 1) reduced the stimulus induced efflux of tritium [Fig. 1(b), Table 1] but increased the stimulus induced efflux of ^{3}H-NA [Fig. 2(a)] compared to the untreated. In this case almost no ^{3}H-deaminated catabolites of ^{3}H-NA were found in the superfusate from the stimulation period or in the iris (Fig. 2).

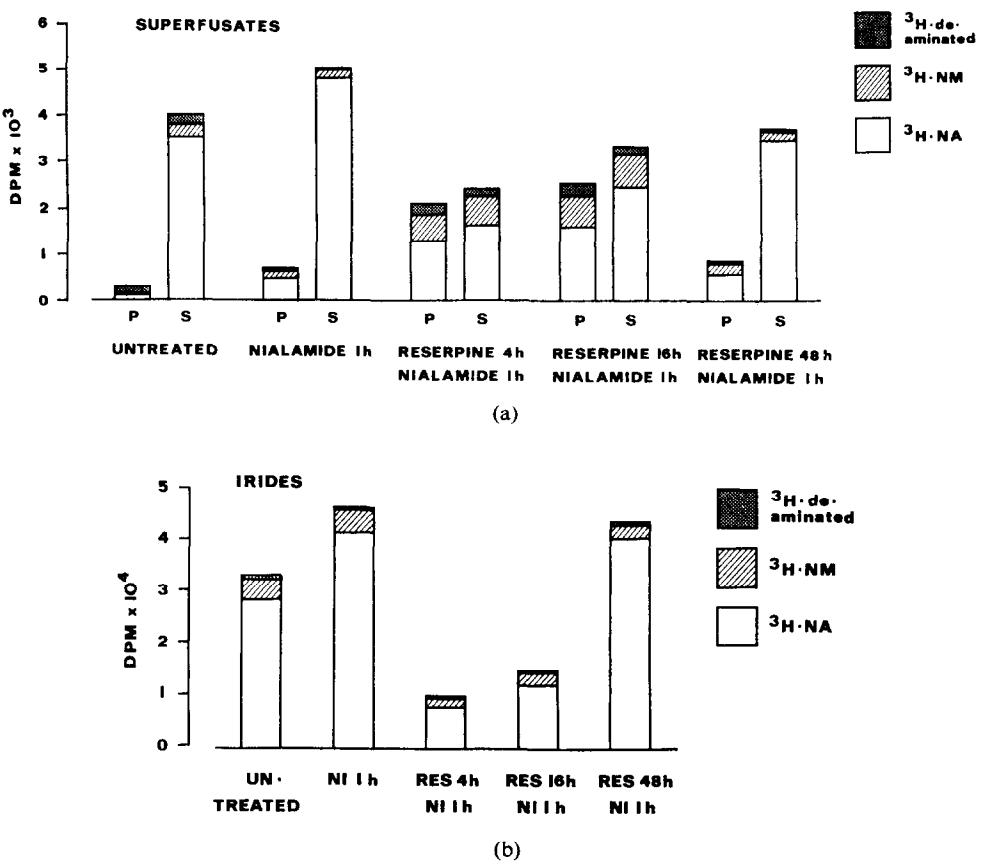

FIG. 2. ^{3}H-NA, ^{3}H-NM and ^{3}H-deaminated catabolites recovered in the superfusate before (P) and during (S) stimulation Figs. 2(a)] and in the iris at the end of superfusion [Fig. 2(b)]. Rats were pretreated as shown in the figures (reserpine 10 mg/kg i.p., nialamide 100 mg/kg i.p.) and isolated irides preincubated with ^{3}H-NA 10^{-7} M. Mean of 4–8 determinations.

TABLE 1. EFFECTS OF RESERPINE AND/OR NIALAMIDE PRETREATMENT ON TISSUE TRITIUM CONTENT AND FIELD STIMULATION INDUCED TRITIUM EFFLUX IN IRIDES INCUBATED WITH ^{3}H-NA*

Treatment	*n*	Total tissue tritium† (dis./min × 100)	Stimulus induced efflux‡ (dis./min × 100)	Per cent stimulus induced efflux§
Untreated	12	560 ± 30	68 ± 5	12·2 ± 0·6
Nialamide 1 hr	8	751 ± 47	55 ± 6	7·3 ± 0·6
Reserpine 4 hr Nialamide 1 hr	7	293 ± 14	2·5 ± 3·2	0·80 ± 0·97
Reserpine 16 hr Nialamide 1 hr	8	305 ± 20	8·9 ± 3·5	2·5 ± 1·0
Reserpine 24 hr Nialamide 1 hr	8	408 ± 36	22 ± 5	5·1 ± 1·1
Reserpine 48 hr Nialamide 1 hr	4	639 ± 44	61 ± 8	9·5 ± 0·6

* Rats were pretreated with reserpine (10 mg/kg i.p.) and/or nialamide (100 mg/kg i.p.) 4–48 and 1 hr respectively before death. Isolated irides were incubated with ^{3}H-NA 10^{-7} M, superfused and stimulated as described in Methods.

† The total tritium content in the iris at the onset of stimulation was calculated by adding the total tritium efflux from the onset of stimulation and the tissue tritium content at the end of superfusion.

‡ Stimulus induced tritium efflux was calculated by subtracting the spontaneous tritium efflux from the total tritium efflux.

§ Stimulus induced tritium efflux as per cent of total tritium content in the iris at the onset of stimulation.

The values given are means ± S.E.M. of *n* observations. The efflux of total tritium from these experiments is shown in Fig. 1.

Four hr after reserpine (plus nialamide 1 hr) there was a high spontaneous efflux of tritium [Fig. 1(c)]. A great proportion of this spontaneous outflow was ^{3}H-NA [Fig. 2(a)]. Almost no effect of stimulation on the tritium efflux was obtained [Fig. 1(c), Table 1]. The spontaneous tritium efflux was high also 16 and 24 hr after reserpine (plus nialamide 1 hr) [Figs. 1(d) and 1(e)]. A slight stimulus induced efflux (2·5 per cent) was found 16 hr after reserpine [Fig. 1(d), Table 1]. Forty-eight hr after reserpine the stimulus induced efflux equalled that from irides treated with nialamide alone [Fig. 1(f)].

Irides incubated with ^{3}H-NA and superfused with reserpine phosphate. Isolated irides were incubated with ^{3}H-NA 10^{-7}M for 30 min and then superfused with fresh buffer for 30 min. Reserpine phosphate was then added to the medium (final concentration 10^{-7} M) and superfusion continued for 30 min before stimulation was started (Fig. 3). The addition of reserpine to the medium caused a gradual increase in the spontaneous outflow of tritium. This increase was due to increased outflow of ^{3}H-deaminated catabolites of ^{3}H-NA (Fig. 4). Stimulation substantially increased the tritium efflux. About 70 per cent of the tritium outflow during stimulation was recovered as ^{3}H-NA.

Irides incubated with ^{3}H-DA. Isolated irides were incubated with ^{3}H-DA 10^{-7} M or 10^{-6} M, superfused and stimulated. Fourteen per cent of the tissue tritium content was overflowing in response to stimulation (Table 2) and the ratio between ^{3}H-DA

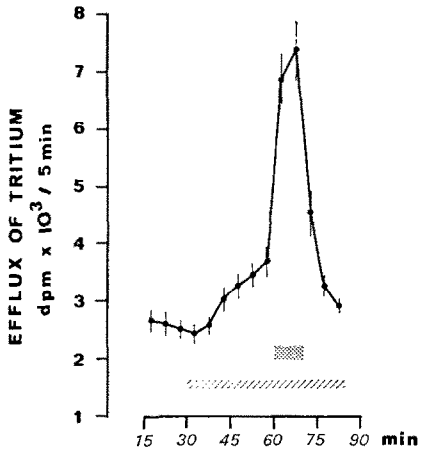

FIG. 3. Effect of reserpine phosphate on tritium efflux. The irides of untreated rats were incubated with ^{3}H-NA 10^{-7} M, superfused with drug-free Krebs–Ringer medium for 30 min and then superfused with reserpine phosphate 10^{-7} M for 30 min before they were stimulated for 10 min.
▨ Reserpine phosphate 10^{-7} M
▩ Stimulation. Mean ± S.E.M. of six experiments.

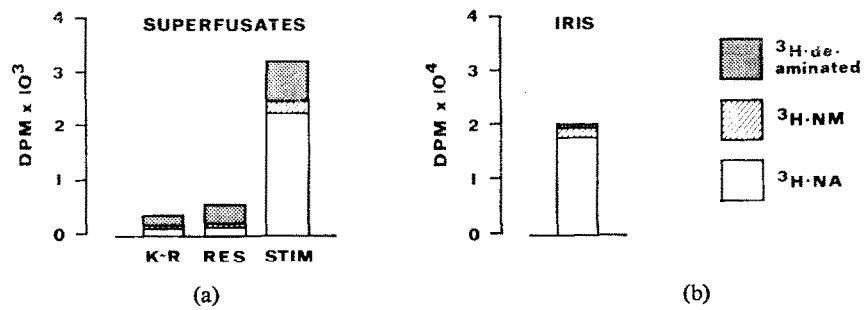

FIG. 4. ^{3}H-NA, ^{3}H-NM and ^{3}H-deaminated catabolites recovered in the superfusate [Fig. 4(a)] and the iris at the end of superfusion [Fig. 4(b)]. The irides of untreated rats were incubated with ^{3}H-NA 10^{-7} M, superfused with drug-free Krebs–Ringer medium (K-R) for 30 min and then superfused with reserpine phosphate 10^{-7} M (RES) for 30 min before they were stimulated (STIM) for 10 min. Mean of six determinations.

and newly formed ^{3}H-NA was 1 to 4 (Fig. 5). About 90 per cent of the labelled amine content in the iris at the end of the superfusion was newly formed ^{3}H-NA. Four hr after reserpine (plus nialamide 1 hr) the stimulus induced efflux was reduced to about 5 per cent and the ratio between ^{3}H-DA and ^{3}H-NA was 2 to 3 (Fig. 5). 70 per cent of the labelled amine content in the iris was newly formed ^{3}H-NA. Sixteen hr after reserpine (plus nialamide 1 hr) stimulus induced efflux was 8 per cent (Table 2).

Irides incubated with ^{3}H-MA. Isolated irides were incubated with ^{3}H-MA 10^{-7} M or 10^{-6} M, superfused and stimulated. The spontaneous tritium efflux was higher after reserpine pretreatment (Fig. 6). Irrespective of the pretreatment stimulation enhanced the efflux. The per cent stimulus induced efflux was about the same in irides of untreated, nialamide or reserpine pretreated rats (Table 3). The ^{3}H-MA content was lower after nialamide or reserpine pretreatment.

TABLE 2. EFFECT OF RESERPINE AND NIALAMIDE PRETREATMENT ON TISSUE TRITIUM CONTENT AND FIELD STIMULATION INDUCED TRITIUM EFFLUX IN IRIDES INCUBATED WITH ^{3}H-DA*

Treatment	*n*	Total tissue tritium† (dis./min × 100)	Stimulus induced efflux‡ (dis./min × 100)	Per cent stimulus induced efflux§
Untreated	8	323 ± 14	46 ± 3	14·2 ± 0·8
Reserpine 4 hr Nialamide 1 hr	11	141 ± 8	6 ± 2	4·5 ± 1·2
Reserpine 16 hr Nialamide 1 hr	6	138 ± 14	11 ± 2	8·2 ± 1·1

* Rats were pretreated with reserpine (10 mg/kg i.p.) and nialamide (100 mg/kg i.p.) 4–16 and 1 hr respectively before death. Isolated irides were incubated with ^{3}H-DA 10^{-7} M, superfused and stimulated as described in Methods.

† The total tritium content in the iris at the onset of stimulation was calculated by adding the total tritium efflux from the onset of stimulation and the tissue tritium content at the end of superfusion.

‡ Stimulus induced tritium efflux was calculated by subtracting the spontaneous tritium efflux from the total tritium efflux.

§ Stimulus induced tritium efflux as per cent of total tritium content in the iris at the onset of stimulation.

The values given are means ± S.E.M. of *n* observations.

DISCUSSION

Transmural stimulation of isolated organs by an electrical field causes responses in the organ which can be attributed to release of endogenous neurotransmitters.[33–35] Such stimulation also induces overflow of exogenous ^{3}H-NA into the superfusing buffer.[20–25]

For the present investigation the isolated rat iris was chosen as a suitable organ. It has a dense and even supply of adrenergic nerves.[6] It is thin and offers good possibilities for diffusion of transmitter from the nerves to the superfusing medium. Previous work[21,22] has shown that very good reproducibility is obtained during field stimulation experiments.

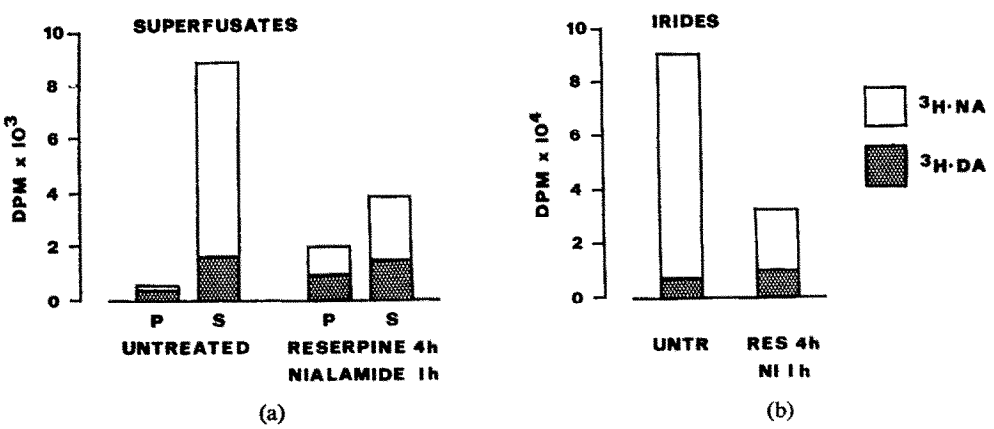

FIG. 5. ^{3}H-DA and ^{3}H-NA recovered in the superfusate before (P) and during (S) stimulation [Fig. 5(a)] and in the iris at the end of superfusion [Fig. 5(b)]. Rats were pretreated as shown in the figures (reserpine 10 mg/kg i.p., nialamide 100 mg/kg i.p.) and isolated irides preincubated with ^{3}H-DA 10^{-6} M.

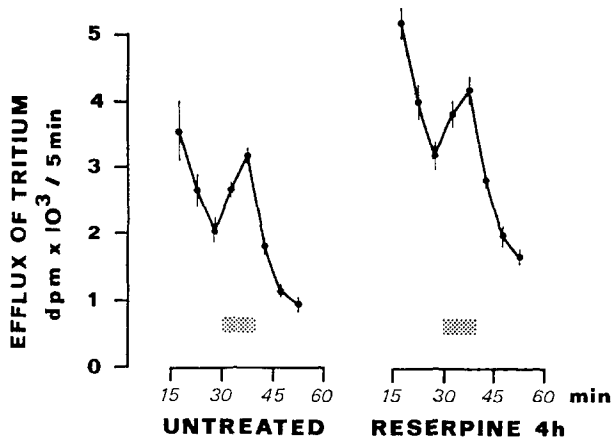

FIG. 6. Efflux of total tritium from isolated irides preincubated with ^{3}H-MA 10^{-6} M. Rats were pretreated as shown in the figure. The dose of reserpine was 10 mg/kg i.p.
▒ Stimulation. Mean ± S.E.M. of four experiments.

The adrenergic nerves in an iris of an untreated rat take up exogenous ^{3}H-NA by the membrane pump and incorporate it into the amine storage granules.[8,12] During subsequent superfusion only a small amount of the spontaneous tritium efflux is recovered as unchanged ^{3}H-NA. A great deal of the radioactivity recovered is ^{3}H-deaminated catabolites. This is in good agreement with other investigations where it has been shown that the greatest part of the spontaneous tritium overflow consists of ^{3}H-deaminated catabolites.[24,25,36]

TABLE 3. EFFECT OF RESERPINE AND NIALAMIDE PRETREATMENT ON TISSUE TRITIUM CONTENT AND FIELD STIMULATION INDUCED TRITIUM EFFLUX IN IRIDES INCUBATED WITH ^{3}H-MA*

Treatment	Concentration of ^{3}H-MA	n	Total tissue tritium† dis./min × 100	Stimulus induced efflux‡ dis./min × 100	Per cent stimulus induced efflux§
Untreated	10^{-7} M	12	244 ± 9	20 ± 1	8·1 ± 0·5
Nialamide 1 hr	10^{-7} M	7	161 ± 17	13 ± 1	8·7 ± 0·7
Reserpine 4 hr	10^{-7} M	4	175 ± 9	14 ± 1	8·0 ± 0·7
Untreated	10^{-6} M	9	852 ± 110	57 ± 8	6·8 ± 0·8
Reserpine 4 hr	10^{-6} M	4	462 ± 37	40 ± 3	8·8 ± 0·8
Reserpine 16 hr	10^{-6} M	8	610 ± 48	50 ± 7	8·0 ± 0·8

* Rats were pretreated with reserpine (10 mg/kg i.p.) or nialamide (100 mg/kg i.p.). Isolated irides were incubated with ^{3}H-MA 10^{-7} M or 10^{-6} M, superfused and stimulated as described in Methods.

† The total tritium content in the iris at the onset of stimulation was calculated by adding the total tritium efflux from the onset of stimulation and the tissue tritium content at the end of superfusion.

‡ Stimulus induced tritium efflux was calculated by subtracting the spontaneous tritium efflux from the total tritium efflux.

§ Stimulus induced tritium efflux as per cent of total tritium content in the iris at the onset of stimulation.

The values given are means ± S.E.M. of n observations.

Field stimulation substantially enhances the overflow of total tritium. The increase in ^{3}H-NA overflow from the prestimulatory period is about 40-fold. The greatest part of the radioactivity recovered is ^{3}H-NA. About 6 per cent is recovered as ^{3}H-deaminated catabolites. Since the recovery of ^{3}H-deaminated catabolites is likely to be lower than that of ^{3}H-NA and ^{3}H-NM (see Methods) the true figure for ^{3}H-deaminated catabolites is probably two or three times higher.[23]

Nialamide pretreatment lowers the stimulus induced efflux of tritium, while the efflux of unchanged ^{3}H-NA is increased compared to the untreated.[37] The increased overflow of unchanged ^{3}H-NA is probably due to inhibited deamination of released ^{3}H-NA. Almost no ^{3}H-deaminated catabolites are found in the superfusate. The reduced stimulus induced efflux of tritium caused by nialamide may be due to several reasons, see further discussion below.

Reserpine effectively blocks the Mg^{2+}-ATP dependent uptake mechanism of the amine storage granules.[9–12] Exogenous ^{3}H-NA, taken up into the nerves after pretreatment with reserpine, is localized mainly extragranularly in the axoplasm provided that MAO has been inhibited.[4,6,16] A reserpine-resistant uptake of ^{3}H-NA into the granules can be demonstrated[16,17,38] but is quantitatively of little importance *in vitro*.[17] The spontaneous efflux of tritium and unchanged ^{3}H-NA is strongly increased 4, 16 and 24 hr after reserpine (plus nialamide 1 hr). The most probable explanation for the impaired retention is the extragranular localization of the amine.[6,8,39] Almost no stimulus induced efflux is obtained 4 hr after reserpine. The stimulus induced efflux increases 16 and 24 hr after reserpine and 48 hr after reserpine it exceeds the value after nialamide pretreatment only. At the same time the capacity of the nerves to retain the accumulated amine is increased.[40] However, the magnitudes are not the same, as the tissue tritium content is doubled, while the stimulus induced tritium efflux increases more than 20-fold. The present results are thus in agreement with the earlier findings that extragranularly located NA cannot be released by nerve stimulation[41] or field stimulation.[21]

Addition of reserpine to the buffer during superfusion of an iris preincubated with ^{3}H-NA leads to an increased outflow of ^{3}H-deaminated catabolites. ^{3}H-NA spontaneously leaking from the amine storage granules cannot be taken up again into the granules but is catabolized by MAO.[42] Stimulation can still cause a markedly increased overflow of unchanged ^{3}H-NA. This is in line with *in vivo* findings that response of the effector organ upon stimulation after reserpine is obtained until endogenous NA is exhausted.[43] Thus, blockade of the Mg^{2+}-ATP dependent mechanism by reserpine does not prevent release of granularly located ^{3}H-NA.

Adrenergic nerves incubated with ^{3}H-DA take up the amine by the membrane pump and incorporate it into the amine storage granules via the Mg^{2+}-ATP dependent mechanism. In the granules ^{3}H-DA is converted to ^{3}H-NA by the enzyme DA-β-hydroxylase.[9,31] The stimulus induced efflux is 14 per cent. This figure is very similar to that found in irides preincubated with ^{3}H-NA. After preincubation with ^{3}H-DA the main amine overflowing in response to stimulation is ^{3}H-NA.

Reserpine inhibits the synthesis of ^{3}H-NA from ^{3}H-DA by inhibiting the uptake of ^{3}H-DA into the storage granules.[9,31,44] The synthesis inhibition can partly be counteracted by nialamide which by inhibition of MAO makes extragranular accumulation of ^{3}H-DA possible. In this case ^{3}H-DA can to some extent gain access to the DA-β-hydroxylase via a reserpine-resistant uptake mechanism and be converted to

^{3}H-NA.[14,31,45] Four hr after reserpine (plus nialamide 1 hr) substantial amounts of ^{3}H-NA are formed from ^{3}H-DA in isolated irides. The stimulus induced efflux is 4 per cent and the ratio between ^{3}H-DA and ^{3}H-NA is 2 to 3. These findings are in agreement with the view of Malmfors[41] and Häggendal and Malmfors[45] that exogenous DA, taken up into the adrenergic nerves after reserpine and nialamide, can be incorporated into the amine storage granules by a reserpine-resistant mechanism, converted to NA and subsequently released by stimulation.

MA is taken up into the adrenergic nerves, incorporated into the amine storage granules[26,27] and released by nerve stimulation.[28] MA is not attacked by MAO and can accumulate in the adrenergic nerves also after reserpine. It can furthermore be incorporated into the amine storage granules via a reserpine-resistant mechanism.[14,15] Field stimulation causes release of ^{3}H-MA. The stimulus induced efflux is 8 per cent and is not decreased by pretreatment with nialamide or reserpine. The stimulus induced efflux is lower than that from irides of untreated rats preincubated with ^{3}H-NA, but similar to that from irides of nialamide pretreated rats preincubated with ^{3}H-NA. Nialamide *per se* does not seem to affect the release process as the stimulus induced efflux of ^{3}H-MA is not affected by nialamide. The decreased stimulus induced efflux from irides preincubated with ^{3}H-NA is probably an effect of the MAO-inhibiting action of nialamide. The occurrence of extragranularly located amine may interfere with the release. Furthermore, a certain extragranular uptake of ^{3}H-amine diminishes the granular fraction available for release under the present experimental conditions. It is also probable that the decreased catabolism after nialamide can lead to an increased reuptake and retention of released ^{3}H-NA and thereby a diminished stimulus induced efflux.

The ^{3}H-MA retained in irides of nialamide pretreated rats is less than in irides of untreated rats. Displacement of ^{3}H-MA by monoamines accumulating in the adrenergic nerves after inhibition of MAO may be the explanation for this.[46]

Reserpine 4 or 16 hr reduces the amount of ^{3}H-MA retained in the nerves probably due to inhibition of the Mg^{2+}-ATP dependent mechanism in the amine storage granules. The ^{3}H-MA taken up and retained after reserpine is overflowing in response to stimulation to the same extent as from irides of untreated rats. The present results confirm recent findings by Almgren and Lundborg.[47]

CONCLUSIONS

Exogenous ^{3}H-NA, ^{3}H-DA (and from ^{3}H-DA formed ^{3}H-NA) and ^{3}H-MA taken up into the adrenergic nerves of isolated rat iris can be released by field stimulation. Reserpine unequally affects the release of the three amines:

1. ^{3}H-NA which is mainly extragranularly located cannot be released.
2. ^{3}H-DA, which is partly taken up into the amine storage granules and converted to ^{3}H-NA, can to a certain extent be released together with newly formed ^{3}H-NA.
3. ^{3}H-MA which is taken up into the granules via a reserpine-resistant mechanism can be released in spite of reserpine.

It is likely that the differences between the three amines are quantitative but not qualitative in nature. It seems that granular localization of the transmitter, regardless of the mechanism for uptake into the granules, makes the transmitter available for

release by stimulation. The results support the view that granular localization is obligate for transmitter release from adrenergic nerves.

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