^{3}H-METARAMINOL RELEASING ACTION OF MESCALINE FROM RAT HYPOTHALAMUS IN VITRO

OM D. GULATI * and NANDKUMAR S. SHAH **

Ensor Foundation Research Laboratory, William S. Hall Psychiatric Institute, P.O. Box 119, Columbia, South Carolina 29202, U.S.A.

Received 2 December 1976, revised MS received 29 April 1977, accepted 2 August 1977

O.D. GULATI and N.S. SHAH, *^{3}H-Metaraminol releasing action of mescaline from rat hypothalamus in vitro*, European J. Pharmacol. 46 (1977) 135—145.

The amine releasing action of mescaline was investigated in rat isolated hypothalamus labeled with ^{3}H-metaraminol. Mescaline had no effect on the uptake of ^{3}H-metaraminol but produced its release in a concentration-related manner. 4×10^{-4} M mescaline, which produced submaximal effects was used for subsequent experiments. 3×10^{-5} M cocaine had no effect on the ^{3}H-metaraminol releasing action of mescaline. Mescaline was fully effective in Ca^{2+}-free medium while 6×10^{-2} M KCl was ineffective. 3×10^{-7} M tetrodotoxin or 6×10^{-5} M lidocaine partially blocked mescaline-induced release but substantially or completely blocked 3×10^{-2} M KCl-induced release. Prior exposure of hypothalamus to 3×10^{-4} M tyramine reduced the releasing action of mescaline. Thus, mescaline appears to release ^{3}H-metaraminol both by Ca^{2+}-independent (tyramine-like) and Ca^{2+}-dependent (lidocaine-sensitive) mechanisms. 3×10^{-4} M tyramine and 6×10^{-2} M KCl released ^{14}C from control hypothalamus labelled with ^{14}C-mescaline, but not from reserpinized hypothalamus. The amounts of ^{14}C recovered in ^{14}C-mescaline labeled control and reserpinized hypothalamus at the end of 50 min of efflux were similar suggesting a poor retention of ^{14}C-mescaline by storage particles.

Mescaline Rat hypothalamus ^{3}H-Metaraminol release

1. Introduction

d-Amphetamine can release noradrenaline (NA) from rat cerebral cortical slices partly by Ca^{2+}-dependent and partly by Ca^{2+}-independent processes (Ziance et al., 1972). The Ca^{2+}-independent catecholamine-releasing action of d-amphetamine and the ability of this drug to act as a substrate for dopamine-β-hydroxylase (Goldstein et al., 1964) would suggest an intraneuronal action of amphetamine. Like d-amphetamine, mescaline is a phenylethylamine and the two drugs also share common behavioral activity (Uyeno and Mitoma, 1969; Sparber and Tilson, 1972). Furthermore, mescaline is taken up by rat cerebral slices and synaptosomes (Gulati and Shah, 1975; Bevan, 1975). The possibility suggested itself that some of the actions of mescaline like those of d-amphetamine (Creese and Iversen, 1972; Carlsson, 1970) could be due to the release of the catecholamine.

After the intraventricular administration of ^{3}H-NA in rats, the accumulation of labeled amine in the various brain regions correlates with distribution of endogenous NA, highest levels being found in the hypothalamus (Glowinski and Iversen, 1966). ^{3}H-Metaraminol which is a phenylethylamine, mimics the effects of NA in the peripheral nervous system (Trendelenburg et al., 1962) and can be taken up, stored and released as a false transmitter by adrenergic nerve endings in the central nervous system (Carlsson and Lindqvist,

* Present address: Department of Pharmacology, Medical College, Baroda, Gujrat, India.
** Reprint requests should be sent to the attention of N.S. Shah at the above address.

1962; Shore et al., 1964). Mescaline mimics the effects of NA on single cortical neurones of cat (Bevan et al., 1974). Mescaline is taken up by rat brain in vitro (Gulati and Shah, 1975; Bevan, 1975) and releases NA from it in vivo (Stolk et al., 1974). It, therefore, appeared interesting to see if mescaline could release ^{3}H-metaraminol from rat brain labeled with this amine. Thus in the present study, the possible ^{3}H-metaraminol releasing action of mescaline and the mechanism thereof have been examined in the rat hypothalamus. Data were also obtained on the release of ^{14}C-mescaline.

2. Materials and methods

2.1. Preparation of hypothalamic slices and incubation

Sprague—Dawley rats (250—350 g) of both sexes were sacrificed by decapitation. All subsequent dissection procedures were performed in the cold room (4°C). The whole brain was carefully removed and placed on filter paper moistened with Krebs bicarbonate medium of the following composition (mM); NaCl 94.11; KCl 4.69; $CaCl_2$ 2.52; $MgSO_4 \cdot 7H_2O$ 0.48; KH_2PO_4 1.17; $NaHCO_3$ 25.0 and dextrose 11.1 (pH 7.4). Hypothalamus was dissected according to the procedure of Glowinski and Iversen (1966) and sliced into right and left halves with the two halves taken as a single preparation (mean weight, 93.3 ± 1.5 mg; n = 110). The tissue was held in position by an envelope (2 cm × 1.5 cm) of fine nylon meshwork attached to the lower end of an oxygenating tube. The nylon meshwork together with the tissue was immersed in 3 ml of the Krebs medium contained in glass vials which were serially arranged and secured in a Dubnoff Metabolic Shaker set at 37°C. The oxygenating tube not only served to gas the medium, but also to transfer the tissue to the series of glass vials at specified intervals. Krebs bicarbonate medium used for incubation and washout was pregassed with a mixture of 95% O_2—5% CO_2 and was continuously bubbled during the experiment. After preincubation for 15 min, the tissue was transferred to the next vial containing either 2 μCi ^{3}H-metaraminol or 3 μCi ^{14}C-mescaline and 300 μg unlabeled mescaline (final mescaline concentration, 5×10^{-4} M) and incubated for 30 min.

When measuring the ^{3}H-metaraminol accumulation into the hypothalamus, the slices were removed from the medium at the end of the 30 min incubation period, dipped into a vial containing non-radioactive solution for 2—4 sec, gently rolled on a glass surface to remove adhering solution and weighed. The effect of mescaline on the accumulation of ^{3}H-metaraminol was studied by incubation of the tissue with 4×10^{-4} M mescaline together with ^{3}H-metaraminol.

2.2. Washout of ^{3}H-metaraminol or ^{14}C-mescaline

At the end of the incubation, the tissue was transferred serially to 5 vials each for 5 min periods and subsequently through a series of 25—30 vials each for 1 min periods. The tissue was exposed for 1 min to medium containing mescaline, tyramine or KCl 30, 38 or 46 min after the start of washout. Modified Krebs media containing different concentrations of KCl were prepared by substituting equimolar amounts of KCl for NaCl. The concentrations used for KCl and tyramine were determined by preliminary experiments in which complete concentration-response curves were obtained. 3×10^{-2} M and 6×10^{-2} M KCl which produced 63.5% and 79.2% of the maximum effects respectively and 3×10^{-4} M tyramine and 6×10^{-4} M tyramine which produced 71.4 and 100% of the maximum effects respectively were used. In some experiments, the medium contained cocaine (3×10^{-5} M), tetrodotoxin (3×10^{-7} M) or lidocaine (6×10^{-5} M) during the entire washout period. The effect of Ca^{2+}-lack on the efflux of radioactivity was studied by omitting Ca^{2+} from the medium and by adding to it disodium ethylenediamine-tetra-acetic acid (3×10^{-5} M). Ex-

periments were also performed in which the tissue was pre-exposed to tyramine for 1 min and then transferred every 1 min for 7 min to vials containing normal medium followed by exposure either to mescaline, KCl or tyramine.

2.3. Analytical procedures

1 ml aliquots of incubation or washout media were added to 10 ml of scintillation solution (Bray, 1960) and the radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation spectrometer using ^{133}Ba as an external standard. The counting efficiency for ^{3}H ranged between 25—32% and that for ^{14}C between 80—88%.

To determine the hypothalamic concentrations of labeled amines, the tissue was weighed and then homogenized in 30 volumes of 0.4 M perchloric acid and centrifuged. For ^{3}H-metaraminol 0.5 ml portions of supernatant were placed in a scintillation vial containing 0.1 ml of 5 N NaOH and 0.5 ml of 0.5 M borate buffer (pH 9). To this was added 10 ml of scintillation solution and the samples were counted as above. For ^{14}C-mescaline, the supernatant was adjusted to pH 5.8 with 5 N potassium carbonate solution (Shah and Himwich, 1971); the salt of potassium perchlorate was removed by centrifugation and 0.5 ml aliquot was used for count of radioactivity.

2.4. Reserpine treatment

Reserpine was administered in a dose of 5 mg/kg s.c. 18 h before sacrifice.

2.5. Calculation of efflux of radioactivity

The ^{3}H or ^{14}C content of the tissue at various times during the efflux was calculated by adding successively, in the reverse order, the amount of radioactivity released into each vial during the efflux period to that remaining in the tissue at the end of experiment. The fractional release was calculated as:

$$\frac{\text{radioactivity released in the medium during a given period}}{\text{radioactivity in the tissue at the beginning of that period}}$$

Since cocaine increased the spontaneous efflux of ^{3}H-metaraminol (see Results) the agonist-induced release was calculated as the % of pre-agonist release in all the experiments.

The 30 min tissue accumulation of mescaline was calculated as the sum of radioactivity released during the entire washout period plus that remaining in the tissue at the end of the experiment.

2.6. Drugs

The following drugs were used: tyramine hydrochloride, mescaline hydrochloride (Sigma Chemical Company, St. Louis, Mo.); cocaine hydrochloride (Merck and Company, Inc., Rahway, N.J.); reserpine (Sandril, Eli Lilly and Company, Indianapolis, Ind.); tetrodotoxin (Calbiochem, La Jolla, Ca.); lidocaine hydrochloride (Astra, Worcester, Mass.); disodium ethylene diamine tetra-acetic acid (EDTA; J.T. Baker Chemical Company, Phillipsburg, N.J.); d,l-7-^{3}H-metaraminol (spec. act. 6.72 Ci/mmol) and 8-^{14}C-mescaline (spec. act. 10.0 mCi/mmol) (New England Nuclear Corporation, Boston, Mass.).

2.7. Statistics

Statistical analyses of the data were performed by Student's two-tailed *t*-test.

3. Results

3.1. Spontaneous efflux of ^{3}H-metaraminol

The spontaneous efflux of ^{3}H-metaraminol from slices labeled with this amine was fairly constant (fig. 1). The control spontaneous efflux over the 1-min period preceding the addition of agonists was 0.027 ± 0.0013 (n = 41), while that in the presence of 3×10^{-7} M tetrodotoxin, 6×10^{-5} M lidocaine, 3×10^{-5} M cocaine or Ca^{2+}-free medium was 0.0245 ± 0.0022 (n = 8; $p > 0.05$), 0.0243 ± 0.0023 (n = 4; $p > 0.05$); 0.0346 ± 0.0015 (n = 11; $p < 0.05$) and 0.0249 ± 0.0015 (n = 15; $p > 0.05$) respectively.

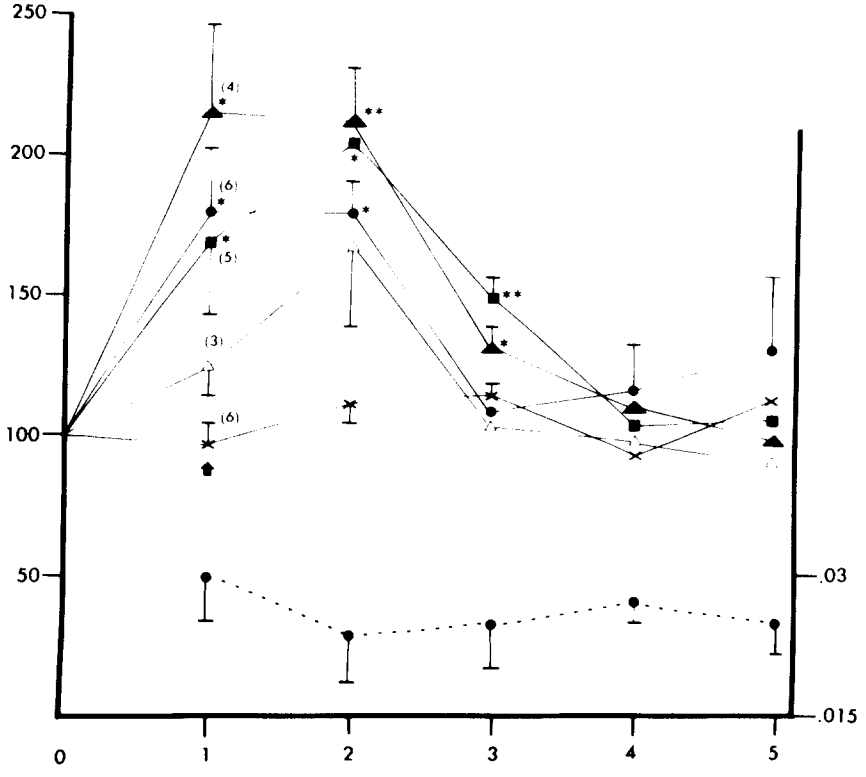

Fig. 1. Fractional release of radioactivity (ordinates) from rat hypothalamus labeled with ^{3}H-metaraminol. Ascissa depicts time in min; 0 time in this and subsequent figures is 29 min after uptake and washout. Right ordinate depicts spontaneous release only. The dpm (mean ± S.E.M.) released at 1, 2, 3, 4 and 5 min were 2150 ± 365, 1779 ± 397, 1873 ± 398, 1715 ± 225 and 1413 ± 188 respectively. Left ordinate depicts mescaline-induced release expressed as % of its own 1-min pre-mescaline release. The tissue was exposed to different concentrations of mescaline added at the begining of the 1 min period. Shown are the mean ± S.E.M. values with the number of experiments in parentheses. The significance of each point was compared with the point for pre-mescaline release and is depicted by * ($p < 0.05$) or ** ($p < 0.01$). Abscissa: time (min). Spontaneous release, ●- - - - -●. Mescaline: 2×10^{-4} M, x———x; 3×10^{-4} M, △———△; 4×10^{-4} M, ●———●; 6×10^{-4} M, ■———■; 8×10^{-4} M, ▲———▲.

3.2. *^{3}H-metaraminol release by mescaline*

2×10^{-4} M concentration of mescaline had no effect on the efflux of radioactivity. Though 3×10^{-4} M mescaline produced a release of ^{3}H-metaraminol, the effect was not significant ($p > 0.05$) when compared with the pre-mescaline spontaneous release. 4×10^{-4} M to 8×10^{-4} M mescaline produced concentration-related release of ^{3}H-metaraminol (fig. 1). The effects of 6×10^{-4} M and 8×10^{-4} M lasted for 3 min and were not significantly different ($p > 0.05$) from each other. 4×10^{-4} M concentration of mescaline which produced submaximal effect was used in subsequent experiments.

3.3. *Effect of different antagonists and Ca^{2+}-free medium on the release of ^{3}H-metaraminol produced by mescaline, KCl and tyramine*

3.3.1. *Mescaline*

3×10^{-5} M cocaine or Ca^{2+}-free medium had no effect on the releasing action of mescaline (fig. 2). 3×10^{-7} M tetrodotoxin and 6×10^{-5} M lidocaine reduced ($p < 0.05$—0.01) the releasing action of mescaline at the first minute; however, the residual release was

still greater than ($p < 0.01$) the pre-mescaline release. These drugs had no effect ($p > 0.05$) on the releasing action of mescaline at the second minute (fig. 2).

3.3.2. KCl

Ca^{2+}-free medium and tetrodotoxin completely blocked ($p < 0.05$—0.01) the ^{3}H-metaraminol releasing action of 3×10^{-2} M or 6×10^{-2} M KCl (fig. 3a, b). Although 6×10^{-5} M lidocaine also produced considerable block ($p < 0.05$—0.01) of the releasing action of 3×10^{-2} M KCl, the release was still significantly ($p < 0.01$) above the pre-KCl level (fig. 3b).

3.3.3. Tyramine

Lidocaine and tetrodotoxin did not affect the release by 3×10^{-4} M tyramine (fig. 4).

3.4. Effect of prior exposure to tyramine on the ^{3}H-metaraminol releasing action of mescaline

Prior exposure of the slices to 3×10^{-4} M tyramine partially reduced ($p < 0.05$) the releasing action of mescaline at the first minute, the residual release being still greater than ($p < 0.05$) the pre-mescaline level. However, the releasing action of mescaline was completely blocked ($p < 0.01$) at the second minute (fig. 2).

The ^{3}H-metaraminol releasing actions of two consecutive exposures to 3×10^{-4} M tyramine were not significantly ($p > 0.1$) different from each other. When the concentration of tyramine was doubled (6×10^{-4} M), the release produced by second exposure to the

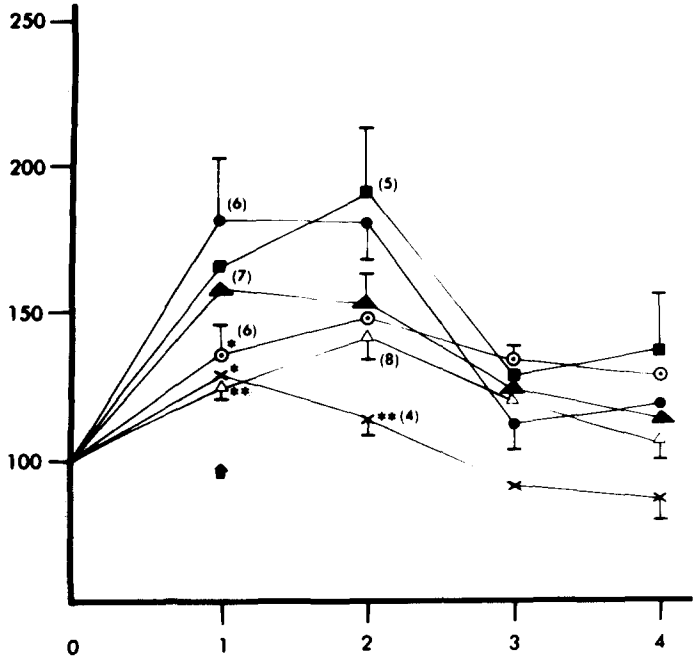

Fig. 2. Fractional release (expressed as % of its own 1-min pre-mescaline release, ordinate) of radioactivity from rat hypothalamus labeled with ^{3}H-metaraminol. The tissue was exposed to 4×10^{-4} M mescaline added at the beginning of the one minute period. Cocaine, Ca^{2+}-free, lidocaine or tetrodotoxin media bathed the tissue during the entire washout period. Pre-exposure to tyramine was made only for 1 min 8 min preceding mescaline addition. Shown are the mean ± S.E.M. values with the number of experiments in parentheses. The significance of each point was compared with the respective control mescaline point and is depicted by * ($p < 0.05$) or ** ($p < 0.01$). Abscissa: time (min). 4×10^{-4} M mescaline: control, ●———●; 3×10^{-5} M cocaine medium, ▲———▲; Ca^{2+}-free medium, ■———■; 6×10^{-5} M lidocaine medium, ⊙———⊙; 3×10^{-7} M tetrodotoxin medium, △———△; pre-exposure to 3×10^{-4} M tyramine, ×———×.

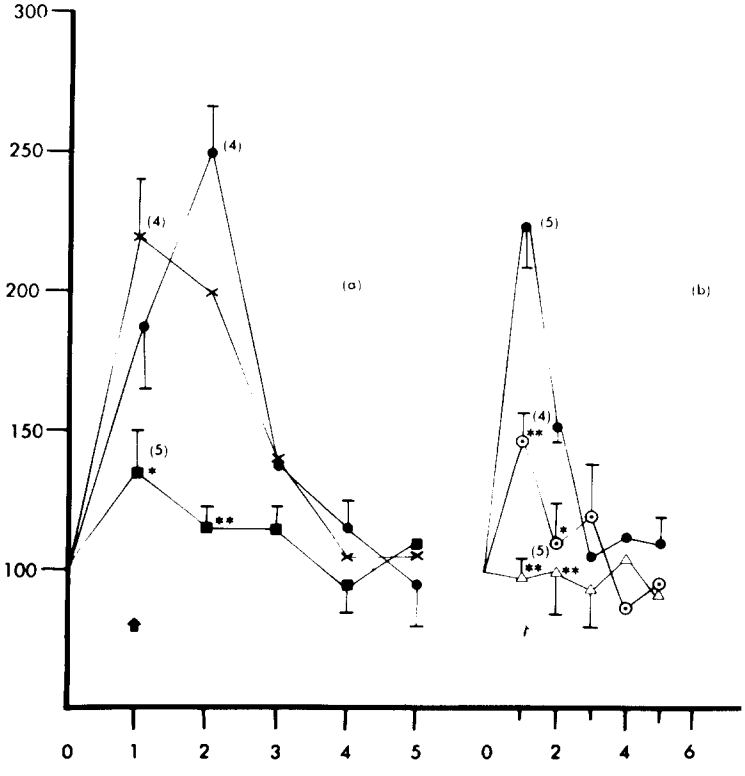

Fig. 3. Fractional release (expressed as % of its own 1-min pre-KCl release, ordinate) of radioactivity from rat hypothalamus labeled with ^{3}H-metaraminol. The tissue was exposed to 6×10^{-2} M KCl (a) or to 3×10^{-2} M KCl (b) added at the begining of the one minute period. Details of Ca^{2+}-free, lidocaine or tetrodotoxin media and pre-exposure to tyramine are given in legend to fig. 2. Shown are the mean ± S.E.M. values with the number of experiments in parentheses. The significance of each point was compared with the respective control KCl point and is depicted by * ($p < 0.05$) or ** ($p < 0.01$). Abscissa: time (min). (a) 6×10^{-2} M KCl: control, ●——●; Ca^{2+}-free medium, ■——■; pre-exposure to 6×10^{-4} M tyramine, ×——×. (b) 3×10^{-2} M KCl: control, ●——●; 6×10^{-5} M lidocaine medium, ⊙——⊙; 3×10^{-7} M tetrodotoxin medium, △——△.

higher concentration of tyramine was significantly ($p < 0.05$) less than that produced by the first exposure (fig. 4). 6×10^{-2} M KCl added 2 min later continued to elicit its releasing action undiminished ($p > 0.1$) (fig. 3a).

3.5. Effect of mescaline on the accumulation of ^{3}H-metaraminol

The tissue level of ^{3}H-metaraminol after incubating it with 1×10^{-7} M of this drug for 30 min was 0.23 ± 0.025 nmoles/g and tissue/medium ratio was 2.7 ± 0.27 (n = 4). The corresponding values obtained in the presence of 4×10^{-4} M mescaline were 0.19 ± 0.018 nmoles/g and 2.14 ± 0.23 (n = 4) and were not significantly different ($p > 0.1$) from the control values.

3.6. Accumulation, release and retention of ^{14}C-mescaline

3.6.1. Control rats

The accumulation of ^{14}C-mescaline calculated from release experiments was 0.26 ± 0.06 μmoles/g (n = 3). The spontaneous efflux of ^{14}C like that of ^{3}H-metaraminol was also fairly constant (fig. 5a). The amount of ^{14}C remaining in the tissue at the end of 50

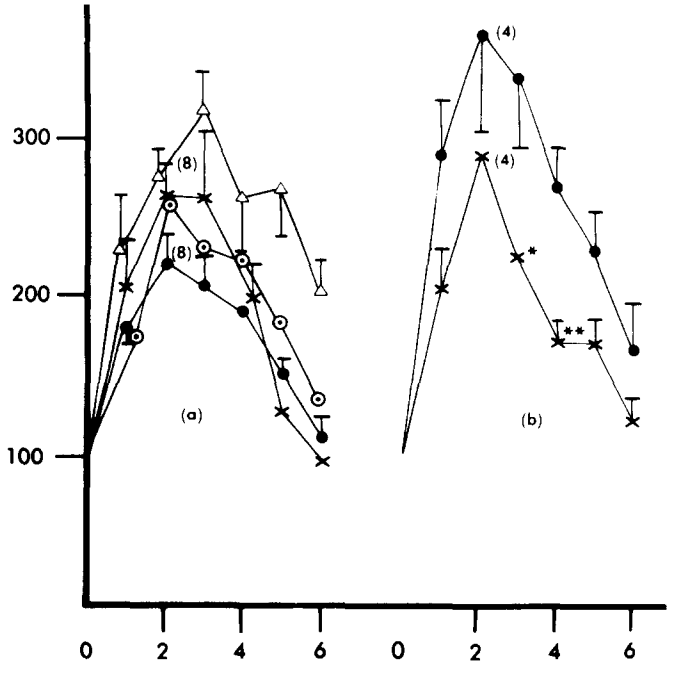

Fig. 4. Fractional release (expressed as % of its own 1-min pre-tyramine release, ordinate) of radioactivity from rat hypothalamus labeled with ^{3}H-metaraminol. The tissue was exposed to 3×10^{-4} M tyramine (a) or to 6×10^{-4} M tyramine (b) added at the beginning of the one minute period. Details of pre-exposure to tyramine are given in legend to fig. 2. Shown are the mean ± S.E.M. values with the number of experiments in parentheses. The significance of each point was compared with the respective control tyramine point and is depicted by * ($p < 0.05$) or ** ($p < 0.01$). Abscissa: time (min). (a) 3×10^{-4} M tyramine: control, ●———●; 3×10^{-7} M tetrodotoxin medium, △———△; pre-exposure to 3×10^{-4} M tyramine, ×———×; 6×10^{-5} M lidocaine medium, ⊙———⊙. (b) 6×10^{-4} M tyramine: control, ●———●; pre-exposure to 6×10^{-4} M tyramine, ×———×.

min of efflux was 0.022 ± 0.001 μmoles/g (n = 3).

Tyramine (3×10^{-4} M) and KCl (6×10^{-2} M) released ($p < 0.01$) ^{14}C at the first minute. A second exposure to the same concentration of tyramine did not have any effect (fig. 5a).

3.6.2. Reserpine-treated rats

The accumulation of ^{14}C-mescaline (0.34 ± 0.02 μmoles/g; n = 3), its spontaneous release (fig. 5b) and the amount of ^{14}C remaining in the tissue at the end of 50 min of efflux (0.028 ± 0.004; n = 3) were not affected ($p > 0.05$) by reserpine treatment.

Tyramine (3×10^{-4} M) and KCl (6×10^{-2} M) had no effect ($p > 0.05$) in the reserpinized tissue (fig. 5b).

4. Discussion

Mescaline induced a concentration-dependent release of ^{3}H-metaraminol. Cocaine had no effect on the releasing action of mescaline. Cocaine inhibits the uptake$_1$ of NA and its congeners into the adrenergic neuron (Iversen, 1973). Thus, the releasing action of tyramine which shares the uptake$_1$ mechanism of NA (Iversen, 1973) is inhibited by cocaine (Westfall and Brasted, 1974). Although mescaline is taken up actively by rat brain synaptosomes (Bevan, 1975) and cerebral slices, this uptake is insensitive to cocaine (Gulati and Shah, 1975). The failure of mescaline to block the accumulation of ^{3}H-metaraminol observed in the present study is in line with

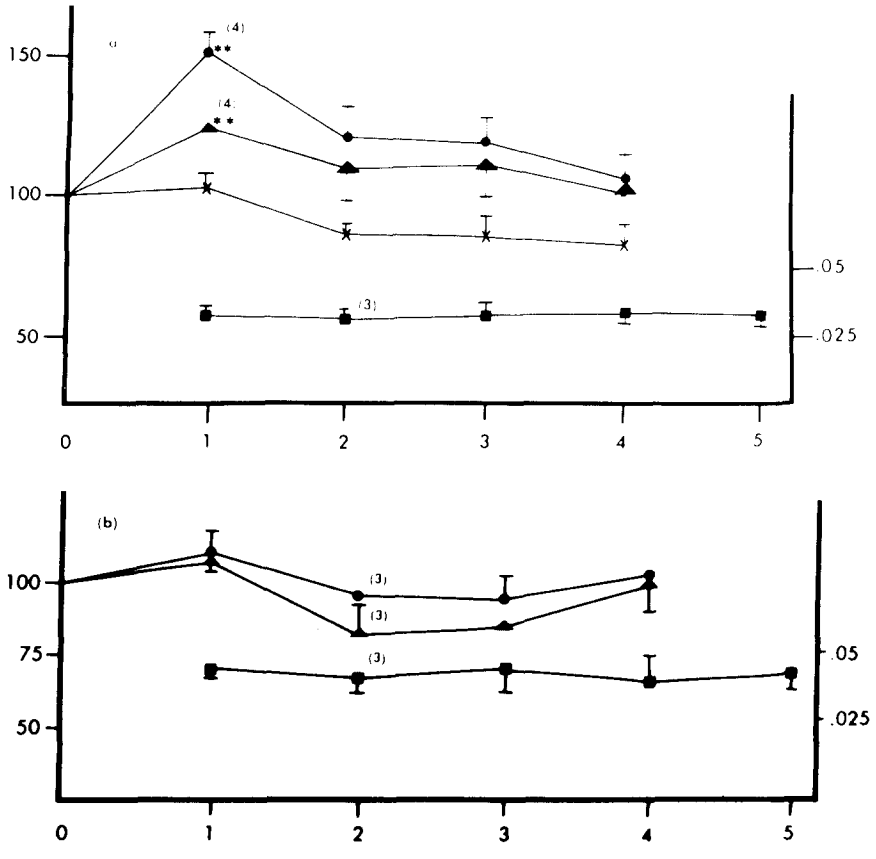

Fig. 5. Fractional release of radioactivity (ordinates) from rat hypothalamus labeled with ^{14}C-mescaline (a, control; b, reserpinized). Right ordinate depicts spontaneous release only. Abscissa depicts time in min; 0 time is 29 min after uptake and washout. The dpm (mean ± S.E.M.) released in normal tissue at 1, 2, 3, 4 and 5 min were 1938 ± 287, 1700 ± 268, 1837 ± 101, 1934 ± 228, and 1522 ± 201 respectively and those in reserpinized tissue were 2692 ± 397, 2642 ± 605, 2482 ± 292, 1924 ± 395 and 1492 ± 407 respectively. Left ordinate depicts release expressed as % of its own 1-min pre-agonist release. The tissue was exposed to 3×10^{-4} M tyramine or 6×10^{-2} M KCl added at the beginning of the 1-min period. Pre-exposure to tyramine was only for 1 min as detailed in legend to fig. 2. Shown are the mean ± S.E.M. values with the number of experiments in parentheses. The significance of each point was compared with the respective point for pre-agonist release and is depicted by ** ($p < 0.01$). Abscissa: time (min). (a) ■———■, Spontaneous release; ●———●, 3×10^{-4} M tyramine; ▲———▲, 6×10^{-2} M KCl; ×———×, 3×10^{-4} M tyramine (pre-exposure to 3×10^{-4} M tyramine). (b) ■———■, Spontaneous release; ●———●, 3×10^{-4} M tyramine; ▲———▲, 6×10^{-2} M KCl.

our previous findings. Had mescaline been a substrate for uptake$_1$, it should have blocked the accumulation of ^{3}H-metaraminol (Iversen, 1973). Block of reuptake of released ^{3}H-metaraminol can, therefore, be excluded as the releasing mechanism of mescaline.

The amine releasing action of nerve stimulation and KCl is either unaffected or potentiated by cocaine (Thoenen et al., 1964; Westfall and Brasted, 1974). In the present study, the ^{3}H-metaraminol releasing action of KCl was absent in Ca^{2+}-free medium, while the releasing action of mescaline was unaffected. Electrical stimulation and KCl induce the release of NA by depolarization of the neuron followed by influx of Ca^{2+} (Hukovic and Muscholl, 1962; Kirpekar and Wakade, 1968). Tetrodotoxin and lidocaine partially blocked the first minute release by mescaline, but had no effect on the second minute release. Tetro-

dotoxin almost completely blocked the releasing action of KCl while lidocaine produced partial but substantial block. Tetrodotoxin and lidocaine block the depolarization of neuronal membrane by blocking the increase in Na^+ conductance (Kao, 1966) and by local anesthetic action respectively. Thus, the absence of releasing action of KCl in Ca^{2+}-free medium and its block by tetrodotoxin and lidocaine are consistent with the mechanism of release by this drug. It is conceivable that mescaline induces the release by mobilizing intracellular Ca^{2+} stores. This suggestion finds support in the reported inhibitory action of local anesthetics on the release of Ca^{2+} from sites to which it is bound in the membrane (Feinstein, 1963). It is also possible that the release of this Ca^{2+} store is in some way related to Na^+ conductance which is blocked by tetrodotoxin (Kao, 1966). Since tetrodotoxin and lidocaine do not affect the uptake of ^{14}C-mescaline (Gulati and Shah, 1977), lesser intraneuronal availability of mescaline could be ruled out. The lack of effect of Ca^{2+}-free medium on the releasing action of mescaline may be attributed to resistance to depletion of intracellular Ca^{2+} stores by this procedure (Van Breemen et al., 1972).

The inability of tetrodotoxin or lidocaine to completely or substantially block the releasing action of mescaline prompted us to examine for additional sites for the releasing action of mescaline, particularly since these drugs had no effect on the ^{3}H-metaraminol releasing action of tyramine. Large doses of tyramine deplete tissue stores of catecholamines (Weiner et al., 1962). It is, therefore, to be expected that tyramine or a drug mimicking the action of tyramine should be less effective or ineffective after an initial exposure to a large dose of tyramine. Prior exposure to 3×10^{-4} M tyramine reduced the releasing action of mescaline at the first minute and completely blocked it at the second minute, but had no effect on the releasing action of second exposure to 3×10^{-4} M tyramine indicating that tyramine released ^{3}H-metaraminol from more than one site and one of these tyramine sensitive sites is affected by mescaline. The releasing action on second exposure to 6×10^{-4} M tyramine (maximal concentration) was less than on first exposure. That the responsivity of the tissue was undiminished in terms of releasable stores of ^{3}H-metaraminol was proved by the ability of KCl administered subsequent to the two successive doses of tyramine to continue to elicit its action unmitigated. These results support the concept of different amine pools involved in the releasing action of tyramine and KCl (Cervoni et al., 1966).

After the accumulation of ^{14}C-mescaline in the tissue, tyramine produced a transient release of ^{14}C-radioactivity. It was ineffective in reserpinized tissue. Since reserpine inhibits "vesicular" uptake of NA (Jonassen et al., 1964), it is possible that mescaline may share this uptake mechanism with NA. The amount of ^{14}C-mescaline released by both tyramine and KCl was less than the amount of ^{3}H-metaraminol released. Moreover, while 3×10^{-4} M tyramine released similar amounts of ^{3}H-metaraminol on two consecutive exposures, this concentration reduced the ^{3}H-metaraminol releasing action of mescaline and released no ^{14}C-mescaline on second exposure. Thus, the accumulation of mescaline into sites where tyramine acts does not appear to be as good as that of ^{3}H-metaraminol. Finally, the amounts of ^{14}C-mescaline recovered in control and reserpinized tissue at the end of 50 min of efflux were similar suggesting a poor "intravesicular" retention of ^{14}C-mescaline, since reserpine affects both granular amine uptake and granular amine storage (Norn and Shore, 1971). Since mescaline lacks both β-hydroxy and phenolic hydroxy groups, which contribute to the efficiency of binding and, therefore, for retention within the storage vesicles (Musacchio et al., 1964, 1966), the poor "intravesicular" retention of mescaline becomes explicable. Because of poor retention, mescaline is not likely to act as a false transmitter.

The results of our study on the ^{3}H-metaraminol releasing action of mescaline which is

partly tyramine-like provide an additional facet to the commonalities of some properties between mescaline and amphetamine (see Introduction). There are, however, some differences between the two drugs, e.g. amphetamine unlike mescaline is an inhibitor of uptake$_1$ (Iversen, 1973) and is substrate for dopamine-β-hydroxylase (Goldstein et al., 1964). While it is not the intent of this paper to minimize these differences, it is possible, that the amine releasing action of mescaline like that of amphetamine may contribute to some of its behavioral effects.

Acknowledgements

This work was supported by Ensor Research Foundation. The authors sincerely thank Alexander G. Donald, M.D., Director, and Joe E. Freed, M.D., Associate Director for Research and Training, William S. Hall Psychiatric Institute for their continued interest in this work. Technical assistance was provided by Mrs. Jannie T. Jones.

References

Bevan, P., 1975, The uptake of mescaline by rat brain synaptosomes, Brit. J. Pharmacol. 54, 257.

Bevan, P., C.M. Bradshaw, M.H.T. Roberts and E. Szabadi, 1974, The effect of microelectrophoretically applied mescaline on cortical neurones, Neuropharmacology 13, 1033.

Bray, G.A., 1960, A simple, efficient liquid scintillator for counting aqeuous solutions in a liquid scintillation counter, Anal. Biochem. 1, 279.

Carlsson, A., 1970, Amphetamine and brain catecholamines, in: International Symposium on Amphetamines and Related Compounds, eds. E. Costa and S. Garattini (Raven Press, New York) p. 289.

Carlsson, A. and M. Lindqust, 1962, In vivo decarboxylation of α-methyl DOPA and α-methyl metatyrosine, Acta Physiol. Scand. 54, 87.

Cervoni, P., S.M. Kirpekar and A. Schwab, 1966, The effect of drugs on uptake and release of catecholamines in the isolated left atrium of the guinea pig, J. Pharmacol. Exptl. Therap. 151, 196.

Creese, I. and S.D. Iversen, 1972, Amphetamine response in rat after dopamine neurone destruction, Nature (New Biology) 238, 247.

Feinstein, M.B., 1963, Inhibition of caffeine rigor and radiocalcium movements by local anesthetics in frog sartorius muscle, J. Gen. Physiol. 47, 151.

Glowinski, J. and L.L. Iversen, 1966, Regional studies of catecholamines in the brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-dopa in various regions of the brain, J. Neurochem. 13, 655.

Goldstein, M., M.R. McKereghan and E. Lauber, 1964, The stereospecificity of the enzymatic amphetamine, β-hydroxylation, Biochem. Biophys. Acta 89, 191.

Gulati, O.D. and N.S. Shah, 1975, Uptake of ^{14}C-mescaline by rat brain cortical slices, Federation Proc. 34, 781 Abs.

Gulati, O.D. and N.S. Shah, 1977, Uptake of ^{14}C-mescaline by rat brain in vitro, (submitted for publication).

Hukovic, S. and E. Muscholl, 1962, Die Noradrenalin Abgabe aus dem isolierten Kaninchen Herzen bei Sympatischer nervenreizung und ihre pharmakologische Beeinflussung, Arch. Exptl. Pathol. Pharmakol. 244, 81.

Iversen, L.L., 1973, Catecholamine uptake processes, Brit. Med. Bull. 29, 130.

Jonassen, J., E. Rosengren and B. Waldeck, 1964, Effect of some pharmacologically active amines on the uptake of arylalkylamines by adrenal medullary granules, Acta Physiol. Scand. 60, 136.

Kao, C.Y., 1966, Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena, Pharmacol. Rev. 18, 997.

Kirpekar, S.M. and A.R. Wakade, 1968, Release of noradrenaline from cat spleen by potassium, J. Physiol. London 194, 595.

Musacchio, J., I.J. Kopin and S. Snyder, 1964, Effects of disulfiram on tissue norepinephrine content and subcellular distribution of dopamine, tyramine and their beta-hydroxylated metabolites, Life Sci. 3, 769.

Musacchio, J.M., J.E. Fisher and I.J. Kopin, 1966, Subcellular distribution and release by sympathetic nerve stimulation of dopamine and alpha-methyl dopamine, J. Pharmacol. Exptl. Therap. 152, 51.

Norn, S. and P.A. Shore, 1971, Further studies on the nature of persistent reserpine binding: evidence for reversible and irreversible binding, Biochem. Pharmacol. 20, 1291.

Shah, N.S. and H.E. Himwich, 1971, Study with mescaline-8-^{14}C in mice: Effect of amine oxidase inhibitors on metabolism, Neuropharmacology 10, 547.

Shore, P.A., D. Busfield and H.S. Alpers, 1964, Binding and release of metaraminol: mechanism of norepinephrine depletion by α-methyl-m-tyrosine and related agents, J. Pharmacol. Exptl. Therap. 146, 194.

Sparber, S.B. and H.A. Tilson, 1972, Tolerance and cross tolerance to mescaline and amphetamine as a

function of central and peripheral administration, Psychopharmacologia 23, 220.

Stolk, J.M., J.D. Barchas, M. Goldstein, W. Boggan and D.X. Freedman, 1974, A comparison of psychomimetic drug effects on rat brain norepinephrine metabolism, J. Pharmacol. Exptl. Therap. 189, 42.

Thoenen, H., A. Huerlimann and W. Haefely, 1964, The effect of sympathetic nerve stimulation on volume, vascular resistance and norepinephrine output in the isolated perfused spleen of the cat and its modification by cocaine, J. Pharmacol. Exptl. Therap. 143, 57.

Trendelenburg, U., A. Muskus, W.W. Fleming and B.G. Alonso De La Sierra, 1962, Modification by reserpine of the action of sympathomimetic amines in spinal cat; a classification of sympathomimetic amines, J. Pharmacol. Exptl. Therap. 138, 170.

Uyeno, E.T. and T.C. Mitoma, 1969, The relative effectiveness of several hallucinogens in disrupting maze performance by rats, Psychopharmacologia 16, 73.

Van Breemen, C., B. Farinas, P. Gerba and E.D. McNaughton, 1972, Excitation contraction coupling in rabbit aorta studied by lanthanum method for measuring cellular calcium flux, Circulation Res. 30, 44.

Weiner, N., P.R. Draskoczy and W.R. Burack, 1962, The ability of tyramine to liberate catecholamines in vivo, J. Pharmacol. Exptl. Therap. 137, 47.

Westfall, T.C. and M. Brasted, 1974, Specificity of blockade of the nicotine-induced release by ^{3}H-norepinephrine from adrenergic neurons of the guinea pig heart by various pharmacological agents, J. Pharmacol. Exptl. Therap. 189, 659.

Ziance, R.J., A.J. Azzaro and C.O. Rutledge, 1972, Characteristics of amphetamine-induced release of norepinephrine from rat cerebral cortex in vitro, J. Pharmacol. Exptl. Therap. 182, 284.