*Biochemical Pharmacology*, Vol. 31, No. 11, pp. 1969–1974, 1982.
Printed in Great Britain.

0006-2952/82/111969-06 $03.00/0


# [$^3$H]DOPAMINE RELEASE BY *d*-AMPHETAMINE FROM STRIATAL SYNAPTOSOMES OF RESERPINIZED RATS

DAVID T. MASUOKA,* ALEJANDO F. ALCARAZ and HERMANN F. SCHOTT
Neuropharmacology Research Laboratory, V. A. Medical Center, Sepulveda, CA 91343, and Medical Pharmacology and Therapeutics, University of California, Irvine, CA 92717, U.S.A.

(*Received* 18 *June* 1981; *accepted* 19 *October* 1981)

**Abstract**—The injection of reserpine, 5 mg/kg i.p. (ipRes), the regimen employed by a majority of investigators, results in synaptosomal and vesicular preparations which are incompletely reserpinized as determined by [$^3$H]dopamine ([$^3$H]DA) accumulation. Reserpine administered by the subcutaneous route, 5 mg/kg (scRes), appears to produce complete reserpinization. Release of [$^3$H]DA by *d*-amphetamine (Amph) was observed from striatal synaptosomes prepared both from normal rats and those pretreated with reserpine intraperitoneally but not from those injected subcutaneously. In the more completely reserpinized scRes synaptosomes, so little [$^3$H]DA had accumulated that release by Amph was not measurable, indicating that if a labile, reserpine-resistant, extravesicular DA *storage* pool releasable by Amph is present under these conditions, it must be extremely small. In scRes monoamine oxidase (MAO)-inhibited preparations, Amph released preloaded [$^3$H]DA located in the cytosol in the absence of functional vesicles. Although chromatographic analysis of the superfusate from ipRes striatal synaptosomes showed that significant amounts of preloaded [$^3$H]DA were released by Amph, the level of dihydroxyphenylacetic acid was not increased over controls, indicating that Amph releases only DA and not its metabolite and is also acting as a MAO inhibitor. No [$^3$H]DA could be released by Amph from superfused hyposmotically shocked normal or ipRes synaptosomes, suggesting that an intact membrane is required for Amph-induced release.

One important mechanism of *d*-amphetamine (Amph) action is the release of catecholamines (CA) from central monoamine neurons [1–3]. It has been postulated that the released CA come from a labile extravesicular pool. As much as 23% of the total dopamine (DA) in the striatum has been assigned to such a "functional compartment" [4]. The hypothesis of a CA release from a newly synthesized, reserpine-resistant pool has been derived from studies utilizing reserpine to deplete the main CA storage pool and to inhibit vesicular CA uptake, together with a tyrosine hydroxylase inhibitor, or radioactive CA or precursors [3, 5–7].

Although reserpinization blocks the uptake of CA into adrenal medullary granules [8, 9], adrenergic nerve granules [10], and brain synaptic storage vesicles [11], uptake of CA still occurs in reserpinized brain slices [12], central monoamine nerve terminals [13], and striatal synaptosomes [14] and furthermore Amph releases DA from striatal slices [15] or reserpine–nialamide-pretreated rats. This has suggested that uptake into and release from a reserpine-resistant extravesicular pool occur. The present study was initiated to determine the presence and characteristics of any reserpine-resistant storage pool of DA releasable by Amph using as a model synaptosomes prepared from the striata of reserpinized rats and preincubated with [$^3$H]DA.

* Send correspondence to: David T. Masuoka, Ph.D., Neuropharmacology Res. Lab. (151B7), V. A. Medical Center, 16111 Plummer St., Sepulveda, CA 91343, U.S.A.

## METHODS

Male Sprague–Dawley rats (Hilltop Lab Animals, Chatsworth, CA) weighing 265–325 g were used. Synaptosomes were prepared from the brains of rats injected 18–20 hr previously with 5 mg/kg reserpine intraperitoneally or subcutaneously and accordingly have been designated ipRes or scRes synaptosomes respectively.

*Synaptosome preparation and superfusion.* Synaptosomes from the striatum were prepared according to the method of Gray and Whittaker [16]. For whole and ruptured synaptosomes, striatal tissue from five or two rat brains, respectively, was pooled, weighed, and homogenized in 10 vol. of cold 0.25 M sucrose. (Striata from five brains weighed 200–300 mg.) The crude synaptosomal fraction, obtained by differential centrifugation, was layered over a discontinuous gradient consisting of 0.8 M and 1.2 M sucrose and centrifuged at 100,000 *g* for 1 hr to give the synaptosome-rich $P_2B$ fraction used in the present experiments.

The $P_2B$ fraction was diluted with 13.5 vol. of Krebs [17] medium to yield approximately 0.6 mg protein/ml. For incubation and superfusion, 200 mg ascorbic acid and 20 mg/liter EDTA were added to Krebs medium and the pH was adjusted to 7.4 with 0.05 N HCl. This suspension was incubated for 10 min with $2 \times 10^{-7}$ M [$^3$H]DA at 37° in an atmosphere of 5% $CO_2$–95% $O_2$. After incubation, 2 ml of the synaptosomal suspension was passed through a Swinnex filter holder (SX00 02500, Millipore Corp., Bedford, MA) with a 0.8 μm MF Millipore

filter, washed with 5 ml Krebs medium (37°), and connected to the superfusion apparatus. Five fractions were collected before superfusion with Amph was initiated. Fractions (1 ml) were collected at a flow rate of 1 ml/4 min. The radioactivity of the wash, after an equivalent volume and quantity of [$^3$H]DA alone was passed through a Millipore filter, decreased to the background count after 10 ml of Krebs medium.

The superfusion apparatus was similar in principle to that described by Raiteri *et al.* [18]. A Technicon Proportioning Pump (Tarrytown, NY) was used to deliver the gassed perfusion medium into the Swinnex filter holder which was suspended in a 37° water bath and, finally, to a fraction collector (Micro Fractionator, Gilson Medical Electronics, Middleton, WI). The superfusion techinque minimizes the reuptake of released DA [18]. This was confirmed by using the DA uptake inhibitor, benztropine methanesulfonate ($10^{-5}$ M), which did not increase the outflow of [$^3$H]DA above control levels.

*Subfractionation of synaptosomes.* After centrifuging, the synaptosomes were subjected to osmotic shock by resuspending in water for 10 min. A sufficient volume of 1.0 M sucrose solution was added to make 0.32 M, and the disrupted synaptosomes were layered over a density gradient consisting of a continuous gradient of 0.4 M–0.6 M over discontinuous gradients of 1.0 M and 1.2 M (modified from Whittaker and Barker [19]). After centrifugation at 100,000 *g* for 60 min, six layers were removed for counting. According to Whittaker and Barker [19], layer 1 contains the supernatant fraction, layer 2 consists of vesicles, layers 3 and 4 of membrane fragments, layer 5 of residual synaptosomes, and layer 6 of intraterminal mitochondria. In the present study, layer 2 was verified by electron microscopy to consist mainly of vesicles. To check the rupturing and subfractionation procedure, striatal synaptosomes from ipRes rat brains were incubated with [$^3$H]DA, divided into two aliquots, and centrifuged at 12,000 *g* for 20 min. After resuspending the pellet in 5 ml of Krebs medium, centrifuging the suspension, and decanting the wash, one aliquot was exposed to hyposmotic shock (0.06 M sucrose) while the other was suspended in 0.32 M sucrose. When both aliquots were fractionated as described above, and the six layers were removed and counted, it was found that the high radioactivity of the intact synaptosomes (layer 5) of the control was decreased after hyposmotic shock and that the radioactivity in the supernatant fraction and vesicle layers increased (Table 1), indicating that the hyposmotic shock procedure was successful in causing bursting of the synaptosomes and extrusion of their contents.

*Superfusion of synaptosomal subfractions.* Ruptured synaptosomes of subfractions preloaded with [$^3$H]DA were superfused in a Micro-Ultrafiltration System, model 8MC (Amicon Corp, Lexington, MA) containing a VSWP 02500 membrane, 0.025 $\mu$m pore size (Millipore Corp.) for particulates, and a PM10 ultrafiltration membrane with a nominal molecular weight cut-off at 10,000 (Amicon Corp.) for the supernatant fraction or total subfractions. The superfusion medium consisted of 0.672% NaCl, 0.035% KCl, 0.028% $CaCl_2$, 0.029% $MgSO_4 \cdot 7H_2O$, 0.10% glucose, in HEPES* buffer (0.02 M, pH 7.0), gassed with 5% $CO_2$–95% $O_2$. Ten milliliters of this medium was placed in the reservoir compartment, and a suspension of ruptured synaptosomes or subfraction (2–7 ml) was placed in the sample chamber. The whole system was placed in a 0–5° environment, and a superfusion pressure up to 40 psi was applied from a tank of nitrogen gas. The sample volume was reduced to 0.5 to 1.0 ml before perfusion from the reservoir was initiated. When this superfusion medium was exhausted, the contents of a small beaker containing 4 ml of medium with or without Amph was tipped in, and superfusion was then continued without a break or change in driving pressure. The filtrate was collected in 0.65-ml fractions.

Table 1. Subfractionation of intact and ruptured (by hyposmotic shock) ipRes synaptosomes after pre-loading by [$^3$H]DA incubation*

| Layers† | Percent counts | |
|---|---|---|
| | 0.32 M | Shocked |
| 1. Supernatant | 20.9 | 53.3 |
| 2. Vesicles | 9.1 | 28.2 |
| 3. Membranes | 3.5 | 5.0 |
| 4. Membranes | 15.4 | 4.2 |
| 5. Synaptosomes | 32.4 | 3.8 |
| 6. Mitochondria | 18.7 | 5.5 |

* The mean activity layered per 100 mg striatum was 804,700 dpm. Results are means of three experiments.
† According to Whittaker and Barker [19].

*High performance liquid chromatography (HPLC).* For separation of radioactive DA and metabolites, a liquid chromatograph, ALC/GPC-204 with a model 6000A solvent delivery system (Waters Associates, Inc., Milford, MA) was used with a Waters Associates $\mu$Bondapak $C_{18}$, 4 mm i.d. × 30 cm column. The eluent consisted of methanol–water–glacial acetic acid (10:90:1) and 0.005 M heptane sulfonic acid or in some experiments 0.17 M acetic acid [20]. Usually a rate of 1 ml/min was used. Authentic carriers consisting of 0.5 to 5 $\mu$g each of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) were added to the samples to be analyzed, and the effluents were collected for counting in a Packard Tricarb spectrometer.

Total recovery of radioactivity from HPLC analysis was about 90% of which about 10% represented a peak appearing at the solvent front. About half of this radioactive peak was contributed by a volatile substance, presumably water, which was removed by lyophilization. The residue was not identified but the retention time did not match that of norepinephrine (NE) or 3,4-dihydroxyphenethanol. It is most probably an oxidation product of [$^3$H]DA since intentional exposure of DA to conditions promoting auto-oxidation causes its retention time to shift to that of the unknown peak.

* HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

*Protein determination.* One 40- and one 80-μl aliquot were removed for protein analysis before incubating synaptosomes with [3H]DA. The method of Schacterle and Pollack [21] was used for the assay.

*Drugs and chemicals.* Reserpine (Serpasil, Ciba Pharmaceutical Co., Summit, NJ) 5 mg/kg, was injected either i.p. or s.c. 18–20 hr before sacrifice. Phenelzine sulfate (a gift from the Warner–Lambert Research Institute, Morris Plains, NJ), 50 mg base/kg s.c., was injected 2 hr before sacrifice. [3H]Dopamine (3,4-dihydroxyphenyl[2-3H]ethylamine, New England Nuclear Corp., Boston, MA) adjusted to a specific activity of 7.5 Ci/mmole, was used at an incubation concentration of $2 \times 10^{-7}$ M. *d*-Amphetamine sulfate (supplied by Smith Kline & French Laboratories, Philadelphia, PA) and benztropine mesylate (gift from Merck Sharp & Dohme Research Laboratories, West Point, PA) were dissolved in the superfusion medium at a concentration of $10^{-5}$ M, and the solutions were gassed before being pumped through the system. Among the chemicals used as carriers, dopamine hydrochloride, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 3-methoxy-4-hydroxyphenylethylamine hydrochloride were purchased from the Calbiochem–Behring Corp., La Jolla, CA) and 3,4-dihydroxyphenethanol from the Regis Chemical Co., Morton Grove, IL.

## RESULTS

*Preparation of completely reserpinized synaptosomes.* Initial experiments were done to establish that well-reserpinized synaptosomes could be prepared. Table 2 shows the HPLC analysis of normal, ipRes, and scRes synaptosomes after incubation with [3H]DA. It is seen that normal and ipRes synaptosomes contained about 25 and 15 pmoles of unchanged DA/mg protein respectively. On the other hand, scRes synaptosomes contained less than 1 pmole of DA/mg.

From Table 1 it is seen that, when ipRes synaptosomes were subfractionated after incubation with [3H]DA, more than 28% of the counts were associated with the vesicle fraction. After a vesicle fraction had been transferred to a Micro-Ultrafiltration System (see Methods) and superfused with 12 ml of medium, 130,000 dpm of 3H (mean of two experiments) was still bound to it. In contrast, when a solution with the same 3H count as the vesicle fraction was placed on the filter, only 4950 dpm remained after superfusion with 12 ml of medium.

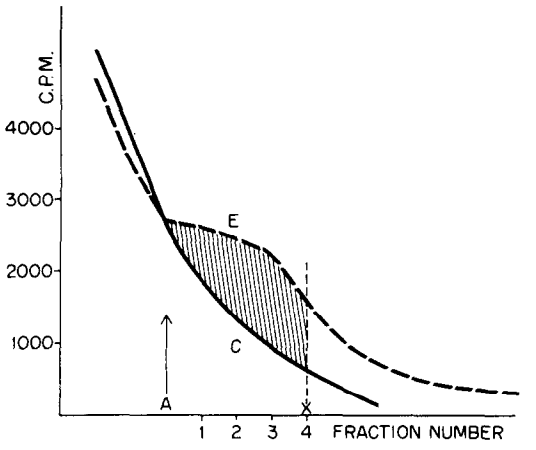


Fig. 1. Superfusion of i.p. reserpinized synaptosomes. At point A, control synaptosomes (C) were exposed to fresh Krebs medium and experimental synaptosomes (E) to Krebs medium containing $10^{-5}$ M *d*-amphetamine, The experiment was terminated at point X. Counts are of 1-ml fractions of the superfusate.

*Amphetamine superfusion of reserpinized synaptosomes.* Figure 1 is a typical superfusion experiment showing the release of 3H-label from ipRes synaptosomes. The release of 3H-label by Amph above the control washout curve is represented by the hatched area. Normal, ipRes, and scRes synaptosomes, after incubation with [3H]DA and superfusion with $10^{-5}$ M Amph, released 3H from normal and ipRes but not from scRes synaptosomes (Table 3). The levels of radioactivity released from normal and ipRes synaptosomes were substantially the same when calculated as percentage of uptake, but that released from ipRes was significantly lower in absolute terms. Release of 3H by Amph was observed even in ipRes synaptosomes from several groups of rats in which all had the appearance of well-reserpinized animals, as characterized by ptosis, hunched posture, diarrhea, and sedation. Several attempts to release [3H]DA from hyposmotically ruptured

Table 2. Analysis of [3H]DA and its metabolites in normal and reserpinized striatal synaptosomes after uptake of [3H]DA and Krebs wash at the point at which *d*-amphetamine would have been added (point A, Fig. 1)

| | Content (pmoles/mg synaptosomal protein) | | |
|---|---|---|---|
| | Normal* | i.p. Reserpine† | s.c. Reserpine‡ |
| DA | 24.85 ±1.40 | 15.83 ± 2.55 | 0.79 ± 0·10 |
| DOPAC | 4.06 ± 0.75 | 6.44 ± 1.29 | 4.31 ± 0.24 |
| HVA | 0.25 ± 0.04 | 0.18 ± 0.08 | 0.13 ± 0.02 |
| 3-MT | 0.15 ± 0.03 | 0.03 | 0.13 ± 0.06 |

* Values are the means ± S.E.M. of four experiments. Each experiment represents the mean of duplicate samples from a pool of synaptsomes from five rat brain striata.

† Values are the means ± S.E.M. of four experiments for all except 3-MT, two experiments (see above).

‡ Values are the means ± S.E.M. of three experiments (see above).

Table 3. Uptake of [$^3$H]DA and release of radioactivity by *d*-amphetamine from normal and reserpinized striatal synaptosomes

| Synaptosomes | Uptake* (dpm/μg protein) | % $^3$H released by *d*-amphetamine† |
|---|---|---|
| Normal (5) | 1086 ± 127 | 24.2 |
| i.p. Reserpine (4) | 436 ± 28 | 21.3 |
| s.c. Reserpine (3) | 103 ± 2 | 0 |

* Mean ± S.E.M. of the radioactivity of synaptosomes as dpm/μg of synaptosomal protein at point A in Fig. 1 of (N) experiments, each representing the mean of duplicate samples from a pool of synaptosomes from five rat brain striata.

† Radioactivity of the amphetamine superfusate minus the radioactivity of the control superfusate divided by the uptake × 100.

Table 4. Analysis of [$^3$H]DA-preloaded ipRes striatal synaptosomes and superfusates after superfusing with or without *d*-amphetamine (values at point X, Fig. 1)

| | Synaptosomal content* | | |
|---|---|---|---|
| | Control (pmoles/mg) | *d*-Amphetamine (pmoles/mg) | Decrease (pmoles/mg) |
| DA | 11.18 ± 1.35 | 6.62 ± 1.24 | 4.56† |
| DOPAC | 2.70 ± 0.59 | 1.73 ± 0.59 | 0.97‡ |
| HVA | 0.13 ± 0.04 | 0.09 ± 0.05 | 0.04 |
| 3-MT | 0.03 | 0.01 | 0.02 |
| | Superfusate content* | | Increase (pmoles/mg) |
| DA | 1.21 ± 0.22 | 6.05 ± 1.07 | 4.84§ |
| DOPAC | 5.72 ± 0.67 | 5.79 ± 0.57 | 0.07 |
| HVA | 0.23 ± 0.05 | 0.27 ± 0.05 | 0.04 |
| 3-MT | 0.04 | 0.04 | 0 |

* Expressed in pmoles/mg of synaptosomal protein representing means ± S.E.M. of four experiments for all except 3-MT, two experiments (see Table 2).

† $P < 0.03$.

‡ $P < 0.005$.

§ $P < 0.01$.

normal or ipRes synaptosomes by Amph in a Micro-Ultrafiltration system were unsuccessful, although release did occur from intact synaptosomes in the same system.

To determine whether only DA itself, or its metabolites as well, were being released by Amph, the superfusates and radioactivity remaining in the ipRes synaptosomes were analyzed by HPLC. It is seen from Table 4 that essentially only unchanged DA was released by Amph into the superfusate. An equivalent decrease in DA was observed in the synaptosomes after Amph superfusion. The DOPAC level in the synaptosomes also was significantly less after Amph but it did not increase in the superfusate.

Neither Amph nor 50 mM KCl caused measurable release of $^3$H from scRes synaptosomes. Since it appears that the absence of release occurred because of the unavailability of DA, the question arises, would release occur from scRes synaptosomes if the level of extravesicular DA were allowed to rise by inhibiting monoamine oxidase (MAO, EC 1.4.3.4)? Tables 5 and 6 show that DA, which was increased 9-fold after pretreatment with a MAO inhibitor, phenelzine, could be released by Amph in spite of the complete inhibition of vesicular uptake by s.c. reserpinization.

Table 5. Analysis of [$^3$H]DA and its metabolites in scRes phenelzine-pretreated striatal synaptosomes after uptake of [$^3$H]DA and Krebs wash at the point at which *d*-amphetamine would have been added (point A, Fig. 1)

| | pmoles/mg protein* |
|---|---|
| DA | 7.35 ± 0.17 |
| DOPAC | 1.22 ± 0.07 |
| HVA | 0.09 ± 0.01 |
| 3-MT | 0.47 ± 0.06 |

* Expressed in pmoles/mg of synaptosomal protein, representing means ± S.E.M. of four experiments (see Table 2).

Table 6. Analysis of [3H]DA-loaded striatal synaptosomes from scRes-phenelzine-pretreated rats after superfusing with or without d-amphetamine (at point X)

| | Synaptosomal content* | | |
|---|---|---|---|
| | Control (pmoles/mg) | d-Amphetamine (pmoles/mg) | Decrease (pmoles/mg) |
| DA | 4.32 ± 1.16 | 1.77 ± 0.40 | 2.55† |
| DOPAC | 0.78 ± 0.14 | 0.45 ± 0.09 | 0.33 |
| HVA | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.01 |
| 3-MT | 0.29 ± 0.07 | 0.21 ± 0.04 | 0.08 |
| | Superfusate content* | | Increase (pmoles/mg) |
| DA | 1.37 ± 0.14 | 3.49 ± 0.41 | 2.12‡ |
| DOPAC | 0.88 ± 0.10 | 0.83 ± 0.08 | −0.05 |
| HVA | 0.11 ± 0.02 | 0.17 ± 0.04 | 0.06 |
| 3-MT | 0.11 ± 0.01 | 0.17 ± 0.04 | 0.06 |

* Expressed in pmoles/mg of synaptosomal protein, representing means ± S.E.M. of four experiments (see Table 2).
† $P = 0.05$.
‡ $P < 0.01$.

## DISCUSSION

Release of preloaded [3H]DA by Amph from synaptosomes in which synaptic vesicle uptake is completely inhibited by reserpine would be an indication that release occurred from an extravesicular site. It was important, therefore, initially to be certain that our synaptosomal preparations were well reserpinized. As is shown in Table 2, 5 mg/kg i.p. appears to produce partially, and s.c. injection more completely, reserpinized preparations. HPLC analysis of radioactivity taken up in the ipRes synaptosomes revealed that nearly 16 pmoles of DA/mg protein was present unmetabolized. This suggests that the [3H]DA taken up was bound to some structures within the synaptosomes, possibly to some vesicles which escaped reserpinization, being thus protected from breakdown by MAO. When ipRes synaptosomes were ruptured by hyposmotic shock after incubation with [3H]DA and subfractionated by density gradient centrifugation, 28% of the total counts was observed to be associated with the vesicle layer (Table 1). Residual radioactivity after superfusion of vesicles indicates that the above counts were mostly from 3H bound to vesicles rather than from contamination by the adjacent supernatant layer. Thus it appears that reserpine, 5 mg/kg i.p., the mode of administration employed by a majority of investigators, results in incomplete, and the s.c. route in more completely, reserpinized vesicles. This result provides evidence at a subcellular level, supplementing the conclusions of Halaris and Freedman [22], that i.p. injections of reserpine produced animals which are only "partially reserpinized" and suggests that interpretations of results in some previous brain studies wihich were based upon data obtained from i.p. reserpinized animals might be subject to reassessment. A preliminary study indicated that a dose of 1 mg/kg s.c. is sufficient to block release while 0.1 mg/kg s.c. produced a result resembling that of ipRes, i.e. 5 mg/kg i.p. A reserpine dose of 15 mg/kg i.p. completely blocked release. The marked difference in effective dosage is probably due to different amounts reaching the brain, presumably as a result of greater exposure to the liver by the i.p. route.

Release of radioactivity by Amph occurred only from the normal and ipRes striatal synaptosomes (Table 3). The low level of [3H]DA accumulated by the scRes synaptosomes (about 3% of normal controls, Table 2) is not released measurably by Amph. These results indicate, assuming that all the DA pools are labeled by [3H]DA, that there is no reserpine-resistant storage pool from which Amph can release DA, and that Amph acts on whatever DA is present in the cytosol that has escaped catabolism by MAO. The possibility that there may not be more than one storage pool has been discussed in detail by Paden [23] and others [24]. It seems that if there are two or more DA storage pools as suggested by McMillen *et al.* [25], they must all be reserpine sensitive and probably reside in the vesicles. This does not preclude the existence of a significant steady-state level or transitory pool of DA in the cytosol of active nerve endings *in vivo*.

No release of radioactivity by Amph could be elicited by superfusing hyposmotically shocked normal or ipRes synaptosomes or certain subfractions of synaptosomes, although unshocked synaptosomes did release under the same conditions. This indicates that either hypotonic shock alters the binding properties of the subfractions or that an intact synaptosomal membrane is required for Amph to induce release of DA into the perfusate. The latter is consistent with the hypothesis that the efflux of CA is an active, carrier-mediated process and, as Amph is transported across the synaptosomal membrane to the inside, the carrier is available for the transport of CA across the membrane to the outside [26, 27].

In spite of a marked increase in the release of DA by Amph from ipRes synaptosomes, the DOPAC did not increase in the perfusate (Table 4), and, in fact, decreased in the synaptosomes (Table 4). This appears to have been the result of MAO inhibition, thus supporting the view that the long known but

generally disregarded MAO-inhibiting property of Amph contributes significantly to its mechanism of action [28–30].

Pretreatment with phenelzine, a MAO inhibitor, increased the level of unchanged [$^3$H]DA 9-fold after uptake by scRes synaptosomes to 46% of that of ipRes synaptosomes (without phenelzine pretreatment) (Table 5). Amph released about 25% of the total radioactivity from scRes synaptosomes containing this increased content of [$^3$H]DA (Table 6). The ability of Amph to release [$^3$H]DA from scRes-phenelzine synaptosomes, in which vesicle uptake was shown to be thoroughly blocked, indicates that functional vesicles were not required for Amph to cause release. The release of [$^3$H]DA located in the cytosol of MAO-inhibited synaptosomes is additional evidence that Amph was acting at the neuronal membrane (see above).

Our experiments do not yield an estimate of the amount of DA which might be present in the cytosol at any one time *in vivo*, but do support the view that the action of Amph *in vivo* depends simultaneously upon (a) a supply of DA to the cytoplasm either from the synaptic vesicle storage pool or from biosynthesis or uptake, (b) the MAO-inhibiting action of Amph which prevents the degradation of the DA thus ensuring its availability in the cytoplasm in a sort of transient pool, and (c) an intact synaptosomal membrane through which Amph stimulates the outward transport of DA via a membrane carrier mechanism.

*Acknowledgements*—We thank Dr. Norio Kokka for helpful suggestions on the manuscript. This work was supported by the Medical Research Service of the Veterans Administration.

## REFERENCES

1. L. Stein, *Fedn Proc.* **23**, 836 (1964).
2. A. Carlsson, M. Lindqvist, A. Dahlstrom, K. Fuxe and D, Masuoka, *J. Pharm. Pharmac.* **17**, 521 (1965).
3. C. C. Chiueh and K. E. Moore, *J. Pharmac. exp. Ther.* **192**, 642 (1975).
4. F. Javoy and J. Glowinski, *J. Neurochem.* **18**, 1305 (1971).
5. A. Carlsson, K. Fuxe, B. Hamberger and M. Lindqvist, *Acta physiol. scand.* **67**, 481 (1966).
6. A. Weissman, B. K. Koe and S. S. Tenen, *J. Pharmac. exp. Ther.* **151**, 339 (1966).
7. M. J. Beeson, A. Cheramy and J. Glowinski, *Eur. J. Pharmac.* **7**, 111 (1969).
8. A. Carlsson, N-A. Hillarp and B. Waldeck, *Acta physiol. scand.* **59** (Suppl. 215), 1 (1963).
9. N. Kirshner, *Science* **135**, 107 (1962).
10. R. E. Stitzel and P. Lundborg, *Br. J. Pharmac.* **29**, 99 (1966).
11. T. A. Slotkin, M. Salvaggio, C. Lau and D. F. Kirksey, *Life Sci.* **22**, 823 (1978).
12. H. J. Dengler, I. A. Michaelson, H. E. Spiegel and E. Titus, *Int. J. Neuropharmac.* **1**, 23 (1962).
13. B. Hamberger and D. T. Masuoka, *Acta pharmac. tox.* **22**, 363 (1965).
14. J. T. Coyle and S. H. Snyder, *J. Pharmac. exp. Ther.* **170**, 221 (1969).
15. L-O. Farnebo, *Acta physiol. scand.* (Suppl. 371), 45 (1971).
16. E. G. Gray and V. P. Whittaker, *J. Anat.* **96**, 79 (1962).
17. H. A. Krebs, *Biochim. biophys. Acta* **4**, 249 (1950).
18. M. Raiteri, F. Angelini and G. Levi, *Eur. J. Pharmac.* **25**, 411 (1974).
19. V. P. Whittaker and L. A. Barker, in *Methods of Neurochemistry* (Ed. R. Fried), Vol. 2, p. 1. Marcel Dekker, New York (1972).
20. L. D. Mell and A. F. Gustafson, *Clin. Chem.* **23**, 473 (1977).
21. G. R. Schacterle and R. L. Pollack, *Analyt. Biochem.* **51**, 654 (1973).
22. A. E. Harris and D. X. Freedman, *Eur. J. Pharmac.* **32**, 93 (1975).
23. C. M. Paden, *J. Neurochem.* **33**, 471 (1979).
24. R. Papeschi, *Psychopharmacology* **55**, 1 (1977).
25. B. A. McMillen, D. C. German and P. A. Shore, *Biochem. Pharmac.* **29**, 3045 (1980).
26. C. O. Rutledge, *Biochem. Pharmac.* **27**, 511 (1978).
27. J. F. Fischer and A. K. Cho, *J. Pharmac. exp. Ther.* **208**, 203 (1979).
28. S. S. Parmar, *Biochem. Pharmac.* **15**, 1497 (1966).
29. H. H. Miller, P. A. Shore and D. E. Clarke, *Biochem. Pharmac.* **29**, 1347 (1980).
30. A. L. Green and M. A. S. El Hait, *J. Pharm. Pharmac.* **30**, 262 (1978).