INITIAL RELEASE OF [³H]DOPAMINE FROM RAT STRIATAL SYNAPTOSOMES: CORRELATION WITH CALCIUM ENTRY¹

PIERRE DRAPEAU² AND MORDECAI P. BLAUSTEIN³

Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Received April 2, 1982; Revised November 22, 1982; Accepted November 22, 1982

Abstract

Calcium entry and dopamine (DA) release were compared in rat striatal synaptosomes, to evaluate the role of Ca in mammalian CNS presynaptic neurotransmission. After equilibrating the synaptosomes with 0.1 μ M [³H]DA, release was measured at 0.1-min intervals by collecting superfusion effluents; release was measured over 1- to 10-sec intervals by a novel rapid filtration technique in which release was stopped by adding a solution containing Ni, a Ca channel blocker. During the initial second, the rate coefficient of high K- and Ca-evoked [³H]DA release ($0.067 \pm 0.003 \text{ sec}^{-1}$) was 40-fold greater than basal release ($0.0017 \pm 0.003 \text{ sec}^{-1}$) and much greater than estimated in previous superfusion studies. Evoked release declined at longer intervals ($0.009 \pm 0.001 \text{ sec}^{-1}$ between 1 and 10 sec), but was still evident after 1 min of depolarization. The fast phase of [³H]DA release, but not subsequent release, showed voltage-sensitive inactivation. A similar time course of Ca uptake was observed, i.e., there was both a fast, inactivating phase and a slow, non-inactivating phase, as described previously in forebrain synaptosomes (Nachshen, D. A., and M. P. Blaustein (1980) *J. Gen. Physiol.* 76: 709-728). Ca-evoked [³H]DA release during 1- and 10-sec incubations increased as the K concentration was raised above 10 mM and levelled off at 75 mM K. The $K_{0.5}$ for Ca of evoked [³H]DA release was 0.2 mM during the fast phase and 0.7 mM for release between 1 and 10 sec. The effects of varying the K concentration (at 1 and 10 sec) and Ca concentration (at 1 sec) on DA release resembled their effects on Ca uptake into forebrain synaptosomes reported previously. Sr and Ba increased the basal rate of [³H]DA release, but Mg, Co, Ni, La, and Cd were ineffective. Sr, Ba, and La substituted for Ca during K-evoked [³H]DA release, whereas the other divalent cations reduced Ca-dependent evoked release. Micromolar concentrations of La and Cd selectively blocked the fast phase of Ca entry and inhibited evoked [³H]DA release in the presence of low (0.2 mM) external Ca concentrations. We conclude that Ca entry is a limiting step for DA release in synaptosomes during the first few seconds of depolarization.

In 1967, Katz and Miledi proposed a "calcium hypothesis" to explain their observations on neurotransmitter release at the frog neuromuscular junction (NMJ). According to this hypothesis, the Ca that enters during nerve terminal depolarization triggers neurotransmitter release. The calcium hypothesis has been confirmed by electrophysiological studies on a number of preparations,

including the squid giant synapse and the vertebrate NMJ (see Llinas, 1977; Martin, 1977).

The small size of the terminals in the mammalian central nervous system precludes intraterminal micro-electrode recording. Nevertheless, studies on brain slices and isolated nerve ending (synaptosome) preparations from mammalian brain (DeRobertis et al., 1962; Gray and Whittaker, 1962) indicate that transmitter release at central synapses is Ca-dependent. Synaptosomes retain many of the functional properties of *in situ* nerve terminals: for example, they can maintain relatively large resting membrane potentials and can synthesize neurotransmitters and release them upon depolarization in the presence of Ca (Blaustein, 1975; Bradford, 1975; Levi and Raiteri, 1976; Blaustein et al., 1977). Synaptosomes may be ideally suited for the study of the molecular events that couple the entry of Ca to neurotransmitter release.

¹ We are very grateful to Dr. D. A. Nachshen for reading the manuscript and for his helpful suggestions and criticisms throughout the course of this study. We thank Ms. Arvette Wilder for preparing the typescript. P. D. is a Postdoctoral Fellow of the Medical Research Council of Canada. This work was supported by National Institutes of Health Grant NS-16106.

² Present address: Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

³ To whom correspondence should be addressed.

We present evidence here that the evoked release of the neurotransmitter dopamine (DA) from synaptosomes is controlled by Ca entry and that release may approach physiologically expected rates during the first few seconds of depolarization.

Synaptosomes prepared from whole brain are heterogeneous in their transmitter content. Therefore, it is useful to examine synaptosomes obtained from specific brain areas enriched in certain transmitter substances. In the present study, we isolated synaptosomes from the corpus striatum; this brain region is enriched in dopaminergic terminals originating from cell bodies in the substantia nigra (see Moore and Bloom, 1978).

Previous studies of the release of DA and other neurotransmitters from synaptosomes have been performed using continuous superfusion techniques (e.g., Raiteri et al., 1978). In typical experiments, released transmitter was assayed after collecting superfusion effluents over time intervals as long as several minutes. Even when shorter time intervals were used (Cotman et al., 1976; Redburn et al., 1976), only a doubling of the rate of transmitter release was observed when synaptosomes were depolarized in the presence of Ca. The validity of synaptosome preparations as model systems for the study of transmitter release has therefore been questioned (e.g., see Kelly et al., 1979). In order to approximate the physiological time course more closely and to examine the effects of more variables, we have developed a rapid filtration method for examining DA release during the first few seconds of depolarization.

In the present study, we correlated DA release from rat striatal synaptosomes (pre-equilibrated with [^3H]DA) with ^{45}Ca entry. We report here that K-stimulated Ca-dependent DA release occurs in two phases with time courses and ionic and voltage sensitivities similar to those of the two respective Ca channels previously described in rat forebrain synaptosomes (Nachshen and Blaustein, 1980). The rate of DA release that we observed during the initial second (about 7% of the stored [^3H]DA) is 2 to 3 orders of magnitude greater than previously reported rates (e.g., Raiteri et al., 1978). Preliminary reports of these observations have been published (Blaustein et al., 1981; Drapeau and Blaustein, 1981).

Materials and Methods

Preparation of striatal synaptosomes

Corpora striata were dissected from rat brains, and synaptosomes were prepared by a modification (Krueger et al., 1979) of the method of Hajos (1975). The P_2 fraction was used, without further purification on a sucrose gradient. The P_2 fraction, in 0.32 M sucrose containing 0.05 mM EGTA, was equilibrated by the gradual addition of 2.5 vol of ice-cold 5K solution. The 5K solution contained (mM): NaCl, 145; KCl, 5; MgCl_2 , 1; glucose, 10; HEPES, 10, adjusted to pH 7.4 at 25°C with NaOH. In both ^{45}Ca uptake and [^3H]DA release experiments (see below), the K concentration in the K-rich solutions (see "Results") was adjusted by isosmotically substituting K for Na. In some experiments choline, rather than K, was substituted for Na in order to test the effect of Na reduction. The diluted synaptosome suspen-

sions were centrifuged at $13,000 \times g$ at 4°C for 5 min, and the pellets were resuspended in 5K solution.

Measurement of ^{45}Ca uptake

^{45}Ca uptake was measured as described by Nachshen and Blaustein (1980). The synaptosome suspension was prewarmed at 30°C for 15 min. Aliquots (55 μl , containing about 250 μg of protein) were then added to equal volumes of 5K solution or, in order to "predepolarize" some samples, 145K solution (i.e., with 145 mM K and only 5 mM Na). Twenty seconds later, 100- μl aliquots of these suspensions were added to equal volumes of 5K or K-rich solutions containing 0.2 mM Ca labeled with ^{45}Ca (3.5 mCi/mmol). ^{45}Ca entry was stopped by rapidly diluting the incubation media with 4 ml of ice-cold, Ca-free 5K "stop solution" (similar to standard 5K, but also containing 1 mM LaCl_3). The diluted samples were filtered by suction through 25-mm diameter glass-fiber filters (grade no. 25, Schleicher and Schuell, Inc., Keene, NH); the filters were washed rapidly with two more 4-ml aliquots of ice-cold stop solution. The ^{45}Ca content of the material trapped on the washed filters was measured by liquid scintillation spectrometry. Protein was measured by the method of Lowry et al. (1951). The data shown are means $\pm$ SEM of quadruplicate determinations.

Measurement of dopamine release

Superfusion method. In order to label the synaptosomes with [^3H]DA, they were incubated for 30 min at 30°C in 5K solution containing 0.1 μM [^3H]DA (100 Ci/mmol), 0.5 mM ascorbate (to prevent oxidation), 0.05 mM pargyline (a monoamine oxidase inhibitor), and 0.1 mM EGTA (to prevent efflux of [^3H]DA due to contaminating Ca; see "Results"). Aliquots of synaptosomes (150 μl containing about 250 μg of protein) were then pipetted between two glass fiber filters (Whatman GF/C, 25 mm diameter, Whatman, Inc., Clifton, NJ). The synaptosomes trapped between the filters were superfused with Ca-free 5K or 75K solutions (4.5 ml/min $^{-1}$, maintained at 30°C) by using a continuous infusion pump (model 600-2-200, Harvard Apparatus Co., Inc., Natick, MA). The two syringes containing superfusion solutions were connected to the filter holder by plastic tubing. Beginning at 0.4 min, effluent samples were collected at 0.1-min intervals in vials placed on a fraction collector (model 272, International Specialties Co., Lincoln, NE). After 0.6 min, most of the unincorporated [^3H]DA was washed off and a low, steady release rate was obtained. To stimulate (depolarize) the synaptosomes, the superfusion solution was changed by switching three-way stopcocks connecting either syringe to the filter holder. The stimulating solutions contained 5K with 2 mM Ca, or 75K with or without 2 mM Ca. Collected samples and filter pads were assayed for radioactivity in liquid scintillation spectrometry. [^3H]DA release is expressed as the mean percentage of radioactivity ($\pm$ SEM) released during each 0.1-min interval, based on the sum of the radioactivity remaining on the filter pads and in all of the samples collected; each time course was performed in triplicate.

Filtration tank method. We also measured [^3H]DA release by using vacuum filtration tanks in which the effluents could be collected. These filtration tanks permit

the collection of a large number of samples (12 per tank), enabling more variables to be tested in each experiment. Furthermore, we developed a method that enabled us to measure [^3H]DA release over time intervals as short as 1 sec (see below).

Glass fiber filters of 25 mm diameter (grade no. 25, Schleicher and Schuell, Inc.) were placed in each well of Amicon VFM-2 filtration tanks (Amicon Corp., Danvers, MA). To each well were added 2 ml of either 5K or, in order to prepolarize the synaptosomes, 75K solutions prewarmed to 30°C. An aliquot of synaptosomes (50 μl containing about 250 μg of protein), equilibrated with [^3H]DA as described above, was pipetted into one of the wells. The synaptosomes were filtered by suction, and 2 ml of 5K or 75K solution were added again to the well and resuspended. This washing procedure was repeated four more times and lasted a total of 40 sec; almost all of the nonincorporated [^3H]DA was removed. A clean collection tube was placed in the tank under the filter of interest, and 2 ml of a stimulating solution (see "Results" for details of composition) was added to the well. Ten seconds later, a vacuum was applied to stop the efflux. The collected samples and respective filter pads were assayed for radioactivity by liquid scintillation spectrometry. In experiments in which the time course of [^3H]DA release was followed, 2 ml of ice-cold 5K solution containing 20 mM NiCl_2 ("stop solution") were added to the wells to stop the [^3H]DA efflux very rapidly (see "Results"); suction was then applied. The results are expressed as the mean percentage of [^3H]DA released ($\pm$ SEM) based on the sum of the radioactivity remaining on the filter pads and in the samples collected; measurements were performed in quadruplicate.

Results

Ca Uptake

Ca uptake into striatal synaptosomes was compared to the Ca uptake into forebrain synaptosomes reported previously (Nachshen and Blaustein, 1980). At a low (5 mM) K concentration, a small uptake of ^{45}Ca into rat striatal synaptosomes was observed (Fig. 1, *open circle*). This indicates that the synaptosomes are somewhat permeable to Ca under nondepolarizing conditions. Increasing the external K concentration stimulated Ca uptake. The 75 mM K-stimulated ^{45}Ca uptake in striatal synaptosomes resembled the K-stimulated Ca influx in forebrain synaptosomes (Nachshen and Blaustein, 1980) in that two phases of Ca uptake could be distinguished. The influx of ^{45}Ca during the initial second was much greater than during the following 9-sec interval of depolarization (Fig. 1, *solid circle*).

Predepolarization of the synaptosomes, by raising the K concentration 20 sec prior to addition of ^{45}Ca , greatly reduced the Ca uptake during the 1st second; this predepolarization did not affect uptake during the following 9-sec interval (Fig. 1, *open triangle*). Thus, the fast (1 sec) phase of Ca uptake in striatal synaptosomes showed voltage-sensitive inactivation, as in forebrain synaptosomes.

In forebrain synaptosomes, the two phases of Ca uptake show markedly different sensitivities to inhibition

Figure 1. Time course of Ca uptake in striatal synaptosomes. Ca uptake was measured in media containing 0.2 mM Ca (with tracer ^{45}Ca) and either 5K (with 145 mM Na + 5 mM K; ○, △) or 75K (with 75 mM Na + 75 mM K; ●, ▲, □). Some solutions also contained 1 μM La (△, ▲). Some synaptosomes were prepolarized in Ca-free 75K containing 0.1 mM EGTA (□). In this and all subsequent figures (unless otherwise noted), the symbols represent the means of four determinations. Error bars indicate $\pm$ 1 SEM where these extend beyond the borders of the symbols.

by La: the apparent inhibitory constant, K_i , for the fast phase is 0.2 μM and for the slow phase is >100 μM (Nachshen and Blaustein, 1980). Figure 1 (*solid triangle*) shows that addition of 1 μM La to the solution containing 75 mM K and ^{45}Ca greatly reduced the fast phase but did not appear to affect the subsequent K-stimulated Ca entry in striatal synaptosomes.

Dopamine Release

The release of DA from striatal synaptosomes was measured after pre-equilibrating the synaptosomes for 30 min at 30°C in a 5K solution containing 0.1 μM [^3H]DA (see "Materials and Methods"). This treatment resulted in accumulation of about 50% of the total [^3H]DA. Since the total intraterminal volume (about 4 $\mu\text{l}/\text{mg}$ of protein; Blaustein and Goldring, 1975) is less than 1% of the volume of the equilibration medium (1 mg of protein/ml), the synaptosomes are capable of considerable DA accumulation (to a >100 -fold internal to external concentration ratio). In the experiments described below, we compared DA release and Ca entry by measuring the time course of [^3H]DA release and the effects of various cations on this process.

Time course of dopamine release

In one type of experiment, the release of [^3H]DA was measured by collecting continuous superfusion samples at 0.1-min intervals (see "Materials and Methods"). Two phases of (high) K-stimulated, Ca-dependent [^3H]DA release were observed. There was an initial large release observed in the first few samples collected when the superfusion fluid was changed from 5K to 75K with 2 mM Ca (Fig. 2, *A and B*, *solid circle*). Release then rapidly declined but remained above the level measured while superfusing with Ca-free 75K (Fig. 2*A* was typical of several other experiments; in Fig. 2*B* the large error of

Figure 2. Superfusion time course of [^3H]DA release. *A*, After washing off unincorporated [^3H]DA for 0.6 min by superfusion with Ca-free 5K solution (+ 0.1 mM EGTA), the superfusion solution was changed in order to "stimulate" the synaptosomes. The stimulating solutions contained 5K solution + 2 mM Ca ($\circ$), or 75K solution without (+ 0.1 mM EGTA; $\triangle$) or with ($\bullet$) 2 mM Ca. *B*, After washing off unincorporated [^3H]DA for 0.6 min by superfusion with Ca-free (+ 0.1 mM EGTA) 5K ($\circ$, $\bullet$) or 75 K ($\triangle$) solution, the synaptosomes were "stimulated" with 75K solution in the absence (+ 0.1 mM EGTA; $\circ$) or presence ($\bullet$, $\triangle$) of 2 mM Ca. The error bars indicate ± 1 SEM for triplicate determinations.

the basal release masked this observation). The fast phase of release was reduced considerably by superfusing synaptosomes with a K-rich solution prior to the addition of Ca (Fig. 2*B*, open triangle); this may indicate that the early phase of release undergoes partial voltage-sensitive inactivation, perhaps because of the partial inactivation of Ca entry (see above).

To determine the effects of a large number of variables on [^3H]DA release in a single experiment, we used filtration tanks in which effluents could be collected by suction (see "Materials and Methods"). Since the delay between application of a vacuum and removal of the stimulating solutions was about 1 sec, we used an ice-cold 5K stop solution to halt [^3H]DA efflux rapidly. The stop solution contained 20 mM Ni; Ni blocks both Ca entry (Nachshen and Blaustein, 1980) and DA release from synaptosomes and does not, itself, support release (see Fig. 7). In control experiments, we assessed the efficacy of the stop solution by measuring the [^3H]DA release evoked by Ca-containing 75K solution and stopped by suction alone, or terminated by addition of stop solution (prior to suction). In a typical experiment (see Fig. 3), [^3H]DA release stopped by suction alone was $9.5 \pm 0.8\%$ after 2 sec, and $16.2 \pm 1.6\%$ after 10 sec of depolarization. When DA release was terminated by adding stop solution 2 sec after depolarization, followed by suction 8 sec later, the release was $9.7 \pm 0.6\%$. These results demonstrate that the stop solution is as effective as suction alone in terminating release at 2 sec, even when application of the vacuum is delayed by 8 sec. Since the stop solution appears to mix with the stimulating solution within 1 sec, this method is useful for measuring release over intervals as short as 1 sec.

Figure 4 shows the time course of [^3H]DA release during a 10-sec interval when release was stopped with Ni. In Ca-free 5K solution (Fig. 4, open circle), very little additional efflux was observed after the initial 1 sec. The

Figure 3. Effectiveness of Ni as a [^3H]DA release blocker. After "stimulating" the synaptosomes by adding 75K solution containing 2 mM Ca, [^3H]DA release was stopped by applying a vacuum 2 sec (open bar) or 10 sec (stippled and solid bars) later. An equal volume of ice-cold 5K solution containing 20 mM NiCl_2 was added to some samples 2 sec after addition of the "stimulating" solution (solid bar).

DA released during the initial second is probably due mostly to bound [^3H]DA that is not removed by the washing procedure. Therefore, the *ordinate intercept*, obtained by extrapolation of the base line release between 1 and 10 sec (Ca-free 5K; Fig. 4, open circle), is assumed to be the zero time release level. With this assumption, the rate coefficient for base line release (from the slope of the Ca-free 5K line) was about 0.0007 sec^{-1} . With 2 mM Ca present in the 5K solution (Fig. 4, solid circle), DA release increased 2- to 3-fold (rate coefficient = $0.0017 \pm 0.0003 \text{ sec}^{-1}$ in 16 experiments). This effect may be due to the leak of Ca into the synaptosomes from 5K solution (see Fig. 1). Therefore, in all experiments shown (Figs. 2 to 9), the synaptosomes were equilibrated and washed with Ca-free solutions in order to avoid depletion of [^3H]DA.

Figure 4. Initial time course of DA release. [^3H]DA release was measured at the times indicated, after adding "stimulating solutions" containing 5K (○, ●) or 75K (△, ▲) without (+ 0.1 mM EGTA; ○, △) or with (●, ▲) 2 mM Ca. Some synaptosomes were prepolarized in Ca-free 75K containing 0.1 mM EGTA (□, ■) and depolarized in 2 mM Ca-containing (■) or Ca-free 75K containing 0.1 mM EGTA (□).

Depolarization by Ca-free 75K solution (Fig. 4, *open triangle*) also evoked a 2- to 3-fold increase in DA release. Similarly, replacement of 70 mM Na by choline increased release by 2- to 3-fold (not shown). This increase may be due to an effect on the Na-dependent DA carrier resulting from the replacement of 70 mM Na by K in the 75K solution (see Raiteri et al., 1978).

When the synaptosomes were depolarized by a 75K solution containing 2 mM Ca, a large increase in the rate of [^3H]DA efflux was observed; this release occurred in two phases (Fig. 4, *solid triangle*), similar in time course to the two phases of Ca entry (see Fig. 1). At the earliest time measured (1 sec), the release rate was increased to $0.067 \pm 0.003 \text{ sec}^{-1}$ (16 experiments). Over the following 9-sec interval, the rate of release declined to $0.009 \pm 0.001 \text{ sec}^{-1}$ (averaged between 1 and 10 sec, in 13 experiments). The fast (1 sec) phase of [^3H]DA release was therefore about 7 times greater than the release observed during the subsequent 9-sec interval and about 40 times greater than the release observed in 5K containing Ca or in Ca-free 75K.

Since the fast phase of Ca entry shows voltage-sensitive inactivation, we examined the effect of prepolarization on [^3H]DA release. The synaptosomes were prepolarized by washing them for about 40 sec with a Ca-free 75K solution. When the same Ca-free 75K solution was used to stimulate DA release (Fig. 4, *open square*), the rate of release was comparable to the rate during Ca-free 75K stimulation after washing with a 5K solution (Fig. 4, *open triangle*). When Ca was present in the 75K-

stimulating solution after prepolarization with Ca-free 75K (Fig. 4, *solid square*), a marked reduction of the fast phase ($59 \pm 3\%$ of control in eight experiments) of [^3H]DA release was observed; however, the release during the subsequent 9 sec did not decline proportionately. The marked decline in DA release subsequent to prepolarization can probably be attributed to the reduced Ca entry.

Effects of cations on dopamine release

To evaluate further the relationship between DA release and Ca entry, we tested the effects of varying the external K and Ca concentrations. The effects of these manipulations on DA release are compared to their previously reported effects on Ca uptake in rat forebrain synaptosomes (Nachshen and Blaustein, 1980). We also compared the effects of a variety of polyvalent cations on [^3H]DA release with the effects of these cations on neurotransmission observed in other preparations.

Effect of varying the external K concentration. The effect of varying the external K concentration (and, thus, the membrane potential; see Blaustein and Goldring, 1975) on [^3H]DA release in the presence of 2 mM Ca was measured at 1 and 10 sec. As can be seen in Figure 5 (*open and solid circles*), [^3H]DA release increased at K concentrations greater than 10 mM and leveled off at 75 mM, the highest concentration tested (the calculated membrane potential change is indicated on the *upper abscissa* scale); the effects were similar at 1 and 10 sec. In a previous study, Ca uptake into rat forebrain synap-

Figure 5. Effect of varying the external K concentration on DA release. [^3H]DA release was measured 1 sec ($\circ$) and 10 sec ($\bullet$) after adding "stimulating solutions" containing 2 mM Ca and the external K concentration ($[\text{K}]_o$) indicated, with $[\text{Na}] + [\text{K}]$ equal to 150 mM. The ordinates are scaled to the release values obtained in solutions containing 5K and 75K, respectively. The lower abscissa scale shows the $[\text{K}]_o$ tested; the upper abscissa scale shows the calculated depolarization due to raising $[\text{K}]_o$, on the assumption that the membrane potential is close to the K equilibrium potential at elevated $[\text{K}]_o$ (cf. Blaustein and Goldring, 1975). Some 75K solutions contained 0.1 mM EGTA and no Ca (1 sec, Δ ; 10 sec, $\blacktriangle$). Others had 70 mM Na replaced by choline instead of K (1 sec, $\square$; 10 sec, $\blacksquare$).

tosomes at 1 and 10 sec showed a similar dependence on the external K concentration (Nachshen and Blaustein, 1980). This parallel increase in Ca uptake and DA release with increasing external K is further evidence that release is limited by Ca entry through Ca-specific channels.

It should be noted that when K was increased above 5 mM in all of the aforementioned experiments, Na was substituted by K. As can be seen in Figure 5 (*open and solid squares*), substitution of 70 mM choline for Na at 5 mM K, in the presence of Ca, gave the same low level of DA release as did the Ca-free 75K solution (Fig. 5, *open and solid triangles*). Thus, the increased Ca-dependent [^3H]DA release in K-rich media is due to depolarization and not to Na replacement. Similarly, replacement of 70 mM Na by choline in the wash solution did not affect the [^3H]DA release subsequently evoked by 75K with Ca (data not shown); i.e., the reduction (inactivation) of release caused by K-rich wash solution was due to prepolarization rather than replacement of Na.

Effect of varying the external Ca concentration. The effect of varying the external Ca concentration on [^3H]DA release in 75K solutions was measured after 1 sec without prepolarization (fast phase) and after 10 sec following prepolarization (slow phase). As shown in Figure 6 (*solid circle*), the fast phase of DA release had an apparent half-maximal saturation for Ca (K_{Ca}) on the order of 0.2 mM (in the presence of 1 mM Mg). This value is similar to the K_{Ca} values (about 0.2 mM) previously

measured during both phases of Ca uptake in rat fore-brain synaptosomes (Nachshen and Blaustein, 1980). However, the apparent K_{Ca} for the slow phase of DA release was larger (about 0.7 mM; Fig. 6, *solid circle*). These results suggest that Ca entry is the rate-limiting step for DA release, especially during the early phase of release.

Effects of other polyvalent cations. The effects of various polyvalent cations on neurotransmission have been well documented in several NMJ preparations (for review, see Ginsborg and Jenkinson, 1975). Most polyvalent cations increase spontaneous (basal) release, although Mg causes a decrease. Sr and, to a lesser extent, Ba are the only divalent cations known to substitute for Ca, rather than block Ca-dependent evoked release. We examined the effects of these and several other polyvalent cations on "basal" [^3H]DA release (at 5K and at 75K in the absence of Ca) and on evoked release (at 75K in the presence of Ca), in order to compare the properties of release in this system with those of the NMJ.

As can be seen from Figure 7, Mg, Ni, Co, and Cd did not support [^3H]DA release in the absence of Ca in a 5K solution (Fig. 7, *open bars*) or a 75K solution (Fig. 7, *stippled bars*). Furthermore, all of these divalent cations reduced the release of [^3H]DA in 75K solutions containing 0.2 mM Ca (Fig. 7, *solid bars*); Ni, Co, and Cd caused complete blockage at the concentrations indicated.

Sr (1 mM) and Ba (1 mM) increased the release of [^3H]DA in Ca-free 5K solutions (Fig. 8, *open bars*) and supported the release evoked by 75K in the absence of Ca (Fig. 8, *stippled bars*); both were as effective as 1 mM Ca in supporting K-stimulated release. Ca (0.2 mM) did not alter the evoked release supported by 1 mM Sr and Ba (Fig. 8, *solid bars*).

At millimolar concentrations, the trivalent cation La substitutes for Ca during evoked release at the NMJ immediately after its application (Blioch et al., 1968), but it blocks evoked release and greatly increases spontaneous quantal release after several minutes of exposure (Heuser and Miledi, 1971; Miledi, 1971). In synaptosomes, 20 μM La (Fig. 8) and 200 μM La (not shown) did not affect [^3H]DA release into Ca-free 5K during a 10-sec exposure. However, these concentrations of La did support DA release into Ca-free 75K solutions; 200 μM La was as effective as 1 mM Ca in supporting release. The evoked DA release in Ca-containing media was unaffected by La.

Effects of low concentrations of La and Cd. Low concentrations of La (e.g., 1 μM) as well as prepolarization, selectively block the fast phase of Ca entry into striatal synaptosomes at Ca concentrations ranging from 0.02 to 2 mM (see Fig. 1 for effect at 0.2 mM Ca; Nachshen and Blaustein, 1980). In order to explore further the dependence of DA release on Ca entry, we tested the effects of 1 μM La on the time course of [^3H]DA release. As can be seen in Figure 9A (*open triangle*), addition of 1 μM La to the Ca-free 75K depolarizing solution did not effect the release of [^3H]DA, in contrast to the action of higher La concentrations (see above). When the synaptosomes were depolarized in the presence of 0.2 mM Ca (Fig. 9A, *solid triangle*), 1 μM La reduced both the early and late phases of release. At 2 mM Ca, 1 μM La blocked

Figure 6. Effect of varying the external Ca concentration on DA release. [³H]DA release was measured at 1 sec (○) without prepolarization and at 10 sec (●) after a 40-sec depolarization with Ca-free 75K solution. In all instances, the “stimulating solution” (75K) contained 75 mM Na + 75 mM K. The external Ca concentrations ([Ca]_o) are indicated on the *abscissa*; the solutions with 0 mM Ca contained 0.1 mM EGTA. The *ordinates* are scaled so that the release values obtained with 0 and 2 mM Ca, respectively, overlap. The curves were drawn to a hyperbolic equation of the form:

$$R = \frac{R_{\max}}{1 + \frac{K_{Ca}}{[Ca]_o}} + R_{\min}$$

where R is the percentage of [³H]DA release at a particular [Ca]_o, $R_{\max}$ is the maximal percentage of release (7% at 1 sec, 19% at 10 sec), K_{Ca} is the [Ca]_o required for half-maximal release (0.2 mM at 1 sec, 0.7 mM at 10 sec), and $R_{\min}$ is the minimal release, observed with 0 mM Ca + 0.1 mM EGTA (2% at both 1 and 10 sec).

Figure 7. Inhibition of DA release by certain divalent cations. [³H]DA release was measured 10 sec after adding “stimulating solutions:” Ca-free 5K (open bars) or 75K (stippled bars) containing 0.1 mM EGTA, or 75K solution with 0.2 mM Ca (solid bars). Some “stimulating solutions” (as indicated under the bars) contained 1 mM Ni or Co, or 0.1 mM Cd; in others, the Mg concentration (normally 1 mM) was increased to 10 mM. The Ni, Co, Cd, and high Mg data were obtained with different synaptosome preparations: the controls (left-hand set of bars) are the averaged values from the four experiments.

Figure 8. Support of DA release by certain polyvalent cations. [³H]DA release was measured 10 sec after adding “stimulating solutions:” Ca-free 5K (open bars) or 75K (stippled bars) containing 0.1 mM EGTA, or 75K solution with 0.2 mM Ca (solid bars). Some “stimulating solutions” also contained 1 mM Sr, 1 mM Ba, or 0.2 mM La, as indicated below the respective sets of bars; all solutions contained 1 mM Mg. The Sr, Ba, and La data were obtained with different synaptosome preparations: the controls (left-hand set of bars) are the averaged values from the three experiments.

the early phase of Ca influx but had no effect on [³H]DA release (not shown). This contrasts with the inhibition of the fast phase of Ca uptake (not shown) and [³H]DA release (Fig. 4) by prepolarization under these condi-

tions. These data imply that the correlation between Ca entry and DA release is complicated when the Ca load is large, or that 1 μM La affects DA release by mechanisms other than by block of the fast Ca channels, or both. The

Figure 9. Effect of low concentrations of La (A) and Cd (B) on the time course of DA release. [^3H]DA release was measured at the times indicated, after adding "stimulating solutions" containing 5K (○, △) or 75K (●, ▲) and 0.2 mM Ca. Some solutions also contained 1 μM La (A: △, ▲) or 10 μM Cd (B: △, ▲).

evoked release supported by higher La concentrations in Ca-free 75K solution (Fig. 5) may indicate that this trivalent cation can enter the terminals to trigger DA release.

Cd blocks Ca entry into rat forebrain synaptosomes in a manner similar to La (D. A. Nachshen, personal communication); that is, low concentrations ($<20 \mu\text{M}$) of Cd specifically block the fast (inactivating) Ca channels, whereas higher concentrations block both types of channels (inactivating and non-inactivating). We have confirmed this effect of Cd on Ca entry into striatal synaptosomes at 0.2 and 2 mM Ca (not shown). The data in Figure 9B show that the effects of 10 μM Cd on [^3H]DA release are similar to those of 1 μM La. Cd did not increase the [^3H]DA release observed during depolarization by Ca-free 75K solution (Fig. 9B, open triangle). However, Cd partially blocked [^3H]DA release during depolarization in the presence of 0.2 mM Ca (Fig. 9B, solid triangle), although the effect was not specific for the fast phase of release; 10 μM Cd had no effect on [^3H]DA release at 2.0 mM Ca (not shown).

Discussion

Temporal resolution of dopamine release: Use of a rapid quench technique

To be physiologically relevant, measurements of transmitter release from synaptosome preparations ideally require time resolution on the order of a few milliseconds. To date, measurements of DA release from striatal syn-

aptosomes have been performed with superfusion techniques over intervals of several minutes (e.g., Raiteri et al., 1978). In the present report, we describe a rapid filtration technique that enabled us to measure DA release over intervals as short as 1 sec. This interval is 2 to 3 orders of magnitude greater than the normal period of nerve terminal depolarization. Nevertheless, this represents a substantial improvement over previous techniques and has enabled us to correlate Ca entry and DA release.

The better time resolution in our experiments is the result of using certain Ca channel blockers (Ni and, in more recent unpublished experiments, Cd; see Figs. 3 and 7) to terminate DA release rapidly. When measured during the initial second, the release rate (0.07 sec^{-1}) is about 100- to 1000-fold greater than the rate observed over a time interval of 5 min (e.g., Raiteri et al., 1978). It may even be possible to shorten the period of depolarization further by combining this quench technique with the rapid mixing method that has been developed for studying Ca fluxes in synaptosomes (Blaustein et al., 1981; Nachshen, 1982).

Correlation of dopamine release with Ca entry

There are a number of similarities between K-stimulated Ca entry and K-stimulated, Ca-dependent DA release. For example, both processes show a fast (1 sec) phase, which is eliminated by predepolarization, and a slow phase (Figs. 1 and 4). A likely explanation for the rapid decline in the rate of DA release with time, and for the effect of predepolarization, is that the Ca channels inactivate, thereby reducing Ca entry (Nachshen and Blaustein, 1980) and Ca-dependent transmitter release.

Cotman and co-workers (Cotman et al., 1976; Redburn et al., 1976) have previously observed a fast phase of noradrenaline (NA) and γ -aminobutyric acid (GABA) release from synaptosomes during the first few seconds of rapid superfusion. However, the initial release rate was only about twice the rate observed at longer time intervals. The small effect that they observed could be due to the difference in transmitter species examined. However, they employed depolarizing solutions prior to the addition of Ca; thus, the relatively low initial rate of transmitter release could be accounted for by Ca channel inactivation and thus a low initial rate of Ca entry.

Ca entry (Nachshen and Blaustein, 1980) and Ca-dependent DA release (Fig. 5) at 1 and 10 sec show a similar dependence on the external K concentration. The fact that Ca uptake and DA release failed to increase until the K concentration was raised above 10 to 15 mM may indicate that a threshold depolarization is required for activation of the voltage-regulated Ca channels, and thus for DA release. Moreover, the dependence of Ca entry (Nachshen and Blaustein, 1980) and DA release (Fig. 6) on external Ca during the initial second of depolarization are very similar. These findings strongly support the view that Ca entry into nerve terminals plays a key role in evoking DA release. The greater apparent K_{Ca} for Ca during the "slow" phase of DA release (i.e., at 10 sec following predepolarization; see Fig. 6) suggests that factors other than Ca entry may become limiting at longer time intervals. Since unphysiologically large amounts of Ca enter at these long time intervals (see McGraw et al.,

1981), it is not surprising that the external Ca dependence of transmitter release changes as a consequence of, for example, altered intraterminal ion concentrations and vesicle depletion due to a high intraterminal free Ca concentration.

Effects of polyvalent cations

Polyvalent cations exert a variety of effects on neurotransmitter release, depending upon the ionic species: some ions alter basal release, and some ions affect evoked release.

Basal release. In NMJ preparations, Mg decreases spontaneous (basal) quantal release, whereas Co, Ni, Sr, Ba, and especially La increase it (Kita and Van der Kloot, 1973; Ginsborg and Jenkinson, 1976). In synaptosomes, we found that Sr and Ba increased the efflux of [^3H]DA into nondepolarizing media (i.e., "basal release"), whereas Mg, Co, Ni, and Cd had a negligible effect. This is consistent with the permeability of synaptosomes to Sr and Ba under nondepolarizing conditions (Nachshen, 1981). Since intraterminal Ca buffers sequester Sr but not Ba (Rasgado-Flores et al., 1982), Ba may accumulate in the cytoplasm in high concentration and thereby release more [^3H]DA than Ca or Sr under nondepolarizing conditions. The apparent ineffectiveness of the other divalent cations to increase "basal release" could be due to a lack of sensitivity of our measurements over the time intervals tested. However, nonquantal leakage of acetylcholine is far greater than the spontaneous quantal release in vertebrate NMJ preparations (Katz and Miledi, 1977; Miledi et al., 1977; Vyskocil and Illes, 1977; Gorio et al., 1978; Vizi and Vyskocil, 1979). If nonquantal leakage is also the major fraction of basal [^3H]DA release, then effects of divalent cations specific for quantal release may not have been detected.

Evoked release. Several divalent cations, including Mg, Co, and Ni, block Ca-dependent evoked release, and do not substitute for Ca in supporting evoked release at NMJs (Miledi, 1966; Kita and Van der Kloot, 1973; Crawford, 1974; Ginsborg and Jenkinson, 1976). Also, Cd blocks dendritic Ca spikes in mammalian central neurons (Llinas and Sugimori, 1980). These four cations also block depolarization-induced, Ca-dependent release in synaptosomes, as illustrated in the present study of [^3H]DA release and in the study of GABA release by Levy et al. (1974) (the effect of Cd was not tested by Levy et al.).

Sr and, to a lesser extent, Ba are the only divalent cations known to substitute for Ca during evoked release in NMJ preparations (Elmqvist and Feldman, 1965; Miledi, 1966; Dodge et al., 1969). Furthermore, both of these alkaline earth cations permeate synaptosomes through the voltage-gated Ca channels (Nachshen, 1981; Nachshen and Blaustein, 1982). The evoked release of NA and GABA from synaptosomes is also supported by Sr and Ba, and Ba appears to be more effective than either Ca or Sr (Levy et al., 1974; Cotman et al., 1976). In the present study, we found that 1 mM Sr and 1 mM Ba were as effective as 1 mM Ca in supporting K-induced [^3H]DA release.

La can substitute for Ca in supporting cholinergic transmission immediately after its application (Blioch et al., 1968), but release cannot be evoked, even in the presence of Ca, several minutes later (Heuser and Miledi,

1971; Miledi, 1971). In synaptosomes, La is also able to support high K-dependent release of GABA (Levy et al., 1974) and DA (this study).

Correlation of the effects of polyvalent cations on dopamine release with their effects on Ca channels. The agonistic and antagonistic effects of the various polyvalent cations on DA release reported here, as well as on transmitter release in other systems (see above), are consistent with the ability of these cations either to permeate or block, respectively, the voltage-sensitive Ca channels in a variety of preparations (see Hagiwara and Byerly, 1981), including synaptosomes (Nachshen and Blaustein, 1980). Further support for the action of antagonists of Ca-dependent evoked release as Ca channel blockers comes from direct studies of the intraterminal effects of these cations. A variety of cations were introduced into the cytoplasm of frog motor nerve terminals using liposomes as a vehicle (Kharasch et al., 1981). Ca-containing liposomes increased evoked release in the presence of a low external Ca concentration, whereas Mg, Co, and La did not depress Ca-mediated release when applied to the inside of the nerve endings; La caused a dramatic increase in spontaneous quantal release. Thus, the antagonism of evoked release by extracellular Mg, Co, and La occurs only at the external membrane surface, presumably by blocking Ca channels.

Clearly, there is a striking correlation between DA release and Ca entry when the Ca load is small (physiological). The apparent lack of effect of low concentrations of the fast Ca channel blockers, La and Cd, in inhibiting release with large (but not small) Ca loads may be due to the complexity of the events which couple transmitter release to Ca entry, any of which may become rate-limiting over such long time intervals: for example, Ca buffering and vesicle replenishment (see above). Furthermore, if a small amount of Cd enters the terminals, as La apparently does, it may have direct or indirect effects (e.g., by raising the intraterminal free Ca concentration) on the release mechanism when a large Ca load is present. Therefore, we conclude that synaptosomal DA release is limited primarily by Ca entry, but other factors may also be involved when the Ca load is very large (nonphysiological).

Applications of rapid release measurements

Numerous reports, in recent years, have indicated that the release of many neurotransmitters, including DA, is modulated by the presynaptic action of several neuroeffectors (for reviews, see Vizi, 1979; Starke, 1981). Little is known about the mechanisms of presynaptic modulation, but the role of voltage-sensitive Ca channels seems important (see Pellmar, 1981). Therefore, it will be of great interest to determine if fast (phasic), slow (tonic), or both phases of release are modulated, perhaps by regulation of Ca channels. Furthermore, our sensitive measure of transmitter release over time intervals of a few seconds may permit an examination of the properties of release of a variety of transmitters from very small brain areas.

References

- Blaustein, M. P. (1975) Effects of potassium, veratridine and scorpion venom on calcium accumulation and transmitter

- release by nerve terminals *in vitro*. *J. Physiol. (Lond.)* 247: 617-655.
- Blaustein, M. P., and J. M. Goldring (1975) Membrane potentials in pinched-off presynaptic nerve terminals monitored with a fluorescent probe: Evidence that synaptosomes have potassium diffusion potentials. *J. Physiol. (Lond.)* 247: 589-615.
- Blaustein, M. P., N. C. Kendrick, R. C. Fried, and R. W. Ratzlaff (1977) Calcium metabolism at the mammalian presynaptic nerve terminals: Lessons from the synaptosome. In *Society for Neuroscience Symposia. Vol. 2: Approaches to the Cell Biology of Neurons*, W. M. Cowan and J. A. Ferrendelli, eds., pp. 139-160, Society for Neuroscience, Bethesda, MD.
- Blaustein, M. P., D. A. Nachshen, and P. Drapeau (1981) Excitation-secretion coupling: The role of calcium. In *Chemical Neurotransmission: Seventy-five Years*, L. Stjärne, P. Hedqvist, A. Wennmalm, and H. Langercrantz, eds., pp. 125-138, Academic Press, Inc., New York.
- Bloch, Z. L., I. M. Glagoleva, E. A. Lieberman, and V. A. Nenashev (1968) A study of the mechanism of quantal transmitter release at a chemical synapse. *J. Physiol. (Lond.)* 199: 11-35.
- Bradford, H. F. (1975) Isolated nerve terminals as an *in vitro* preparation for the study of dynamic aspects of transmitter metabolism and release. In *Handbook of Psychopharmacology*, L. L. Iversen, S. Iversen, and S. H. Snyder, eds., Vol. 1, pp. 191-252, Plenum Publishing Corp., New York.
- Cotman, C. W., J. W. Haycock, and W. F. White (1976) Stimulus-secretion coupling processes in brain: Analysis of noradrenaline and gamma-aminobutyric acid release. *J. Physiol. (Lond.)* 254: 475-505.
- Crawford, A. C. (1974) The dependence of evoked transmitter release on external calcium ions at very low mean quantal contents. *J. Physiol. (Lond.)* 240: 255-278.
- DeRobertis, E., A. Pellegrino de Iraldi, G. Rodriguez de Lornes Arnaiz, and L. Salganicoff (1962) Cholinergic and non-cholinergic endings in rat brain. I. Isolation and subcellular distribution of acetylcholine and acetylcholinesterase. *J. Neurochem.* 9: 23-35.
- Dodge, F. A., Jr., R. Miledi, and R. Rahamimoff (1969) Strontium and quantal release of transmitter at the neuromuscular junction. *J. Physiol. (Lond.)* 200: 267-283.
- Drapeau, P., and M. P. Blaustein (1981) Biphasic dopamine release from striatal synaptosomes parallels Ca entry. *Soc. Neurosci. Abstr.* 7: 441.
- Elmqvist, D., and D. S. Feldman (1965) Spontaneous activity at a neuromuscular junction in tetrodotoxin. *Acta Physiol. Scand.* 64: 475-476.
- Ginsborg, B. L., and D. H. Jenkinson (1976) Transmission of impulses from nerve to muscle. In *Handbook of Experimental Pharmacology. Vol. 42: Neuromuscular Junction*, E. Zaimis, ed., pp. 229-364, Springer-Verlag, New York.
- Gorio, A., W. P. Hurlbut, and B. Ceccarelli (1978) Acetylcholine compartments in mouse diaphragm. Comparison of the effects of black widow spider venom, electrical stimulation and high concentrations of potassium. *J. Cell Biol.* 78: 716-733.
- Gray, E. G., and V. P. Whittaker (1962) The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.* 96: 79-88.
- Hagiwara, S., and L. Byerly (1981) Calcium channel. *Annu. Rev. Neurosci.* 4: 69-125.
- Hajos, F. (1975) An improved method for the preparation of synaptosomal fractions in high purity. *Brain Res.* 93: 485-489.
- Heuser, J., and R. Miledi (1971) Effect of lanthanum ions on function and structure of frog neuromuscular junctions. *Proc. R. Soc. Lond. (Biol.)* 179: 247-260.
- Katz, B., and R. Miledi (1967) The release of acetylcholine from nerve endings by graded electric pulses. *Proc. R. Soc. Lond. (Biol.)* 167: 23-38.
- Katz, B., and R. Miledi (1977) Transmitter leakage from motor nerve endings. *Proc. R. Soc. Lond. (Biol.)* 196: 59-72.
- Kelly, R. B., J. W. Deutsch, S. S. Carlson, and J. A. Wagner (1979) Biochemistry of neurotransmitter release. *Annu. Rev. Neurosci.* 2: 399-446.
- Kharasch, E. D., A. M. Mellow, and E. M. Silinsky (1981) Intracellular magnesium does not antagonize calcium-dependent acetylcholine secretion. *J. Physiol. (Lond.)* 314: 255-263.
- Kita, H., and W. Van der Kloot (1973) Action of Co and Ni at the frog neuromuscular junction. *Nature New Biol.* 245: 52-53.
- Kreuger, B. K., R. W. Ratzlaff, G. R. Strichartz, and M. P. Blaustein (1979) Saxitoxin binding to synaptosome membranes and solubilized binding sites from rat brain. *J. Membr. Biol.* 50: 287-310.
- Levi, G., and M. Raiteri (1976) Synaptosomal transport processes. *Int. Rev. Neurobiol.* 19: 51-74.
- Levy, W. B., J. W. Haycock, and C. W. Cotman (1974) Effects of polyvalent cations on stimulus-coupled secretion of [¹⁴C]-γ-aminobutyric acid from isolated brain synaptosomes. *Mol. Pharmacol.* 10: 438-449.
- Llinas, R. R. (1977) Calcium and transmitter release in squid synapse. In *Society for Neuroscience Symposia. Vol. 2: Approaches to the Cell Biology of Neurons*, W. M. Cowan and J. A. Ferrendelli, eds., pp. 139-160, Society for Neuroscience, Bethesda, MD.
- Llinas, R., and M. Sugimori (1980) Calcium conductances in Purkinje cell dendrites: Their role in development and integration. *Prog. Brain Res.* 51: 323-334.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 262-275.
- Martin, A. R. (1977) Junctional transmission. II. Presynaptic mechanisms. In *Handbook of Physiology, Section 1: The Nervous System*, Vol. 1, Part 1. J. M. Brookhart and V. B. Mountcastle, section eds., E. R. Kandel, vol. ed., pp. 329-355, American Physiological Society, Bethesda.
- McGraw, C. F., D. A. Nachshen, and M. P. Blaustein (1981) Calcium movement and regulation in presynaptic nerve terminals. In *Calcium and Cell Function*, W. Y. Cheung, ed., Vol. 2, pp. 81-110, Academic Press, Inc., New York.
- Miledi, R. (1966) Strontium as a substitute for calcium in the process of transmitter release at the neuromuscular junction. *Nature* 212: 1233-1234.
- Miledi, R. (1971) Lanthanum ions abolish the "calcium response" of nerve terminals. *Nature* 229: 410-411.
- Miledi, R., P. C. Molenaar, and R. L. Polak (1977) An analysis of acetylcholine in frog muscle by mass fragmentography. *Proc. R. Soc. Lond. (Biol.)* 197: 285-297.
- Moore, R. Y., and F. E. Bloom (1978) Central catecholamine neuron systems: Anatomy and physiology of the dopamine systems. *Annu. Rev. Neurosci.* 1: 129-169.
- Nachshen, D. A. (1981) Influx of Ca, Sr, and Ba, through Ca channels in synaptosomes. *Biophys. J.* 33: 145a.
- Nachshen, D. A. (1982) The initial time-course of Ca influx in synaptosomes. *Biophys. J.* 37: 314a.
- Nachshen, D. A., and M. P. Blaustein (1980) Some properties of potassium-stimulated calcium influx in presynaptic nerve endings. *J. Gen. Physiol.* 76: 709-728.
- Nachshen, D. A., and M. P. Blaustein (1982) The influx of calcium, strontium and barium in presynaptic nerve endings. *J. Gen. Physiol.* 79: 1065-1087.
- Pellmar, T. C. (1981) Transmitter control of voltage-dependent currents. *Life Sci.* 28: 2199-2205.

- Raiteri, M., F. Cerito, A. M. Cervoni, R. del Carmine, M. T. Ribera, and G. Levi (1978) Studies on dopamine uptake and release in synaptosomes. *Adv. Biochem. Psychopharmacol.* 19: 35-56.
- Rasgado-Flores, H., D. A. Nachshen, and M. P. Blaustein (1982) ATP-dependent uptake of Ca, Sr, Ba and Mn by intracellular organelles in synaptosomes. *Soc. Neurosci. Abstr.* 8: 833.
- Redburn, D. A., D. Shelton, and C. W. Cotman (1976) Calcium-dependent release of exogenously loaded γ -amino-[U- ^{14} C] butyrate from synaptosomes: Time course of stimulation by potassium, veratridine, and the calcium ionophore, A23187. *J. Neurochem.* 26: 197-303.
- Starke, K. (1981) Presynaptic receptors. *Annu. Rev. Pharmacol. Toxicol.* 21: 7-30.
- Vizi, E. S. (1979) Presynaptic modulation of neurochemical transmission. *Prog. Neurobiol.* 12: 181-290.
- Vizi, E. S., and F. Vyskocil (1979) Changes in total and quantal release of acetylcholine in the mouse diaphragm during activation and inhibition of membrane ATPase. *J. Physiol. (Lond.)* 286: 1-14.
- Vyskocil, F., and D. Illes (1977) Non-quantal release of transmitter at mouse neuromuscular junction and its dependence on the activity of Na⁺ K⁺ ATPase. *Pflügers Arch.* 370: 295-297.