Br. J. Pharmac. (1976), **58**, 569–572

ON THE FUNCTIONAL COUPLING OF NEUROTRANSMITTER UPTAKE AND RELEASE IN BRAIN

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1 Isolated synaptosomal fractions from mouse forebrains were incubated with [^{14}C]-γ-aminobutyric acid ([^{14}C]-GABA). Release of the accumulated label in high potassium solution was measured.

2 The fractional release dependent upon calcium was decreased by raising the concentration of [^{14}C]-GABA during labelling but was not affected by altering the time allowed for labelling or the time between labelling and stimulation.

3 These data suggest that extracellular GABA gains rapid access to available intraterminal pools. The relative distribution of the accumulated GABA in different pools can be influenced by the concentration of GABA in the incubation medium but, once 'stored', there is no net redistribution of accumulated GABA in the absence of stimulation.

Introduction

Synaptosomes exhibit both high-affinity transport systems for the accumulation of neurotransmitters (Kuhar, 1973; Bennett, Mulder & Snyder, 1974) and Ca-dependent secretion systems for the release of endogenously stored transmitter substances (de Belleroche & Bradford, 1972a,b; Blaustein, Johnson & Needleman, 1972; Cotman, Haycock & White, 1976). It has been proposed that the transport systems serve primarily in the termination of synaptic actions of released transmitters (Iversen, 1971). However, it is also possible that uptake systems can procure transmitter for intraterminal stores that specifically supply readily releasable pools (Hughes, 1973; Hughes & Roth, 1974; Ryan & Roskoski, 1975).

The functional coupling, in synaptosomes, between uptake and calcium-dependent secretion of γ-aminobutyric acid (GABA) and noradrenaline (NA) has been described previously (Levy, Redburn & Cotman, 1973; Levy, Haycock & Cotman, 1974; Cotman, *et al.*, 1976). The secretion processes exhibit properties characteristic of well-studied stimulus-secretion coupling systems, and the accumulation processes that provide transmitter for secretion are Na-dependent. The purpose of the present study was to examine the relationship of transmitter concentration during accumulation and time of accumulation upon the accessibility of newly accumulated transmitter to the release process.

Methods

After homogenization in 0.32 M sucrose, synaptosomal fractions were isolated from forebrains of male Swiss-Webster mice or corpus striata of Sprague-Dawley rats as described previously (Cotman, *et al.*, 1976). Tissue was resuspended in a HEPES-Ringer solution of the following composition (mM): NaCl 140, HEPES 20, glucose 10, K_2HPO_4 1.5, $MgSO_4$ 1.5, and amino-oxyacetic acid 0.1, pH 7.4 with Tris. All subsequent incubation and release manipulations were performed at room temperature (22–24°C). Tissue aliquots (1 ml, 0.6 mg protein) were plated onto filter units mounted on a vacuum box. Filtrates were collected in vials mounted in a sliding plate within the vacuum box (Levy *et al.*, 1974).

Synaptosomal tissue was labelled with 4-amino-*n*-[U-^{14}C] butyric acid (232 mCi/mmol, Amersham/Searle; added to the incubation solution) by perfusing solution through the filter units. After incubation at [^{14}C]-GABA concentrations and labelling durations indicated in the Results section, 2 ml washes were administered to the filter units every 30 s (gravity flowthrough for 20 s; negative pressure, approx. 10 mmHg, for 10 seconds). The wash solution consisted of incubation solution with 50 mM KCl added. After washout with 3 x 2 ml washes, matched filter units received either 2 ml of wash solution or 2 ml of wash solution containing 1.5 mM $CaCl_2$.

Radioactivity in the filters and fourth wash filtrates was analyzed by liquid scintillation spectrometry. Ca-dependent release was calculated as the difference between filtrates (in pmol [^{14}C]-GABA or % efflux) from filter units receiving calcium and filter units (previously treated identically) receiving wash solution with no calcium added during the fourth wash. In some experiments, [^{3}H]-(–)-NA or [^{3}H]-dopamine (New England) were used.

Results

As presented in Table 1, increases in either GABA concentration in the incubation solution or the duration of the labelling period increased the amount of [^{14}C]-GABA accumulated and the pmol of Ca-dependent [^{14}C]-GABA released. When calculated in terms of the fraction of accumulated GABA, however, Ca-dependent [^{14}C]-GABA release was affected only by differences in the incubation concentration of GABA. That is, increases in uptake as a function of incubation duration were paralleled by increases in the amount of [^{14}C]-GABA release due to calcium. On the other hand, the increases in uptake as a function of GABA concentration were only partially accommodated by increases in the amount released. In separate sets of experiments, Ca-dependent release of [^{3}H]-NA from forebrain synaptosomes and [^{3}H]-dopamine from striatal synaptosomes (0.1 mM pargyline included in incubation solutions) also demonstrated a similar dependence upon transmitter concentration during incubation (Table 1).

Kinetic analyses of [^{14}C]-GABA and [^{3}H]-NA accumulation into forebrain synaptosomal fractions and [^{3}H]-dopamine into striatal synaptosomal fractions revealed that even at the highest concentrations investigated in the release studies (10.8, 3.2, 5.0 μM, respectively) the transmitters were accumulated primarily by single, high-affinity sites (GABA: $K_m = 1.4$ μM, $V_{max} = 860$ pmol min^{-1} mg^{-1} protein; NA: $K_m = 2.1$ μM, $V_{max} = 22$ pmol min^{-1} mg^{-1} protein; dopamine: $K_m = 0.3$ μM, $V_{max} = 68$ pmol min^{-1} mg^{-1} protein) under conditions similar to above.

Within the graphical accuracy of Lineweaver-Burk and Eadie-Hofstee analyses, over 80% of the GABA was accumulated via high-affinity sites at the high concentration in Table 1. [^{3}H]-NA and [^{3}H]-dopamine accumulation described kinetically only a single transport site at the concentrations tested. Although not absolutely sensitive in detecting small kinetic differences between transport sites, these graphical measures should have been able to detect uptake via other sites (low affinity) amounting to, e.g., 60% of the total uptake in the case of [^{14}C]-GABA. This would be the extent of low affinity uptake necessary at the higher GABA incubation concentration in order for low affinity (presumably non-readily releasable) accumulation to account for the observed reduction in fractional Ca-dependent release.

Table 1 Calcium-dependent release as a function of incubation condition

Incubation condition					
Concentration (μM)	*Duration (s)*	*Post-labelling period (s)*	*Accumulation* (pmol)*	*Baseline efflux (%)*	*Ca-dependent release (%)*
[^{14}C]-GABA					
0.43	20	600	29 ± 3	1.63 ± 0.11	3.56 ± 0.27
0.43	20	Ø	33 ± 4	3.34 ± 0.35	3.30 ± 0.31
0.43	120	Ø	228 ± 18	2.10 ± 0.18	3.12 ± 0.24
0.43	600	Ø	906 ± 26	1.89 ± 0.06	3.23 ± 0.18
10.8	600	Ø	4350 ± 1010	2.26 ± 0.34	1.31 ± 0.25†
[^{3}H]-NA					
0.06	600	Ø	3.8 ± 0.1	3.50 ± 0.40	6.09 ± 0.64
3.2	600	Ø	79 ± 2	8.34 ± 0.25	2.05 ± 0.14†
[^{3}H]-dopamine					
0.05	600	Ø	49 ± 4	1.71 ± 0.08	9.36 ± 0.40
5.0	600	Ø	297 ± 19	11.65 ± 0.42	4.25 ± 0.31†

* Totals on filter immediately prior to stimulation; calculated on the basis of initial specific activities of labelled transmitter; for [^{14}C]-GABA and [^{3}H]-NA, pmol/0.6 mg forebrain synaptosomal protein and for [^{3}H]-dopamine, pmol/0.3 mg striatal synaptosmal protein.

† $P < 0.05$, two-tailed t-test for inherently paired samples.

All values represent the means and s.e. mean for four determinations.

Discussion

For widely varying amounts of transmitter uptake, fractional release should be diagnostic for variations in the relative distribution of the accumulated transmitter with respect to readily releasable pools (defined here by the 20 s stimulation period). Our findings indicate that incubations as short as 20 s label readily releasable pools in equivalent proportion to incubations up to 30 times longer. Thus the accumulated transmitter not only has access to the readily releasable pools but this access is rapid. Although availability to the release process is rapid, the GABA accumulated during a 20 s incubation did not demonstrate a detectable redistribution with respect to the readily releasable pools over a 10 min period.

Following a 10 min incubation with 0.43 μM [^{14}C]-GABA, % Ca-dependent release of both labelled and endogenous GABA are similar (Cotman, *et al.*, 1976), and the specific activities (sp. act.) of the tissue and released GABA are similar. In that brain GABA levels are relatively insensitive to extra-terminal GABA and amino-oxyacetic acid concentrations *in vitro* (Starr & Tanner, 1975; unpublished observations), alterations of % release of [^{14}C]-GABA for the conditions investigated above should reflect alterations of the released:tissue ratio of GABA sp. act. The decrease in % [^{14}C]-GABA release after incubation at the high GABA concentration suggests: (1) that the sp. act. of the released GABA did not increase in proportion to the increase in tissue GABA sp. act. and (2) that a greater percentage of the labelled GABA (presumably accumulated by the same set of membrane transport sites) was being stored in less readily releasable stores. As such, these data implicate multiple *intra*terminal GABA pools that are kinetically distinct both in terms of (1) affinities and/or storage capacities and in terms of (2) participation in stimulus-secretion coupling processes.

These data are similar to those reported by Hughes (1973) for [^{3}H]-NA release from rabbit vasa deferentia in that higher incubation concentrations produced a greater amount, but lower fraction, of [^{3}H]-NA release. However, in apparent contrast to the present report, the accumulated NA in the vas was released in preference to endogenous NA after incubation at lower NA concentrations. The dynamics of NA in the vas and GABA in the brain may be quite disparate but comparison between these systems is difficult considering the differences in tissue (whole organ *vs* isolated fraction) and temporal aspects (h *vs* min) of the experiments.

Ryan & Roskoski (1975) recently reported that newly accumulated GABA was released in preference to endogenous GABA from isolated synaptosomes. However, the present data showing a constant fractional release over both duration of incubation and incubation-stimulation interval and previous data showing equivalence of tissue and released GABA sp. act. (Cotman, *et al.*, 1976) indicate no such preferential release of accumulated GABA for the conditions described above (notably, without prior stimulation). Ryan & Roskoski (1975) stimulated release by simultaneously elevating K and Ca. We find that elevated K pulses alone (in the absence of Ca) can induce a preferential efflux of labelled GABA from synaptosomes and that without subtraction of this baseline efflux (notably high for K pulses) spurious inferences regarding Ca-dependent release can be made (Cotman, *et al.*, 1976). We have been able to obtain preferential release of accumulated GABA only under conditions of prior stimulation (Levy, Haycock & Cotman, 1976).

These data suggest a physiological role for reuptake processes in maintaining synaptic transmission in brain. The rapidity with which accumulated GABA has access to readily releasable pools makes possible a direct coupling between reuptake and stimulus-secretion coupling processes. The absolute contribution of this reaccumulated transmitter to secretion may be controlled by the extraterminal concentration of transmitter immediately subsequent to release. One hypothesis is that, at higher concentrations, relatively more transmitter is accumulated into those compartments within the terminal where it has a less immediate access to secretory processes (e.g., extravesicular or less readily releasable vesicular stores).

This work was supported by Research Grants NS 08597 to C.W.C. and BMS 75-18089 to W.B.L. and an NSF Predoctoral Fellowship to J.W.H. The data were taken from portions of a doctoral thesis by J.W.H.

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(Received March 30, 1976. Revised June 14, 1976.)