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Exocytotic release of noradrenaline from synaptosomes

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Exocytosis is now generally accepted as the mechanism of secretion of noradrenaline from nerve terminals [1, 2]. Although much is known about the release of neurotransmitters from the autonomic nervous system (ANS), the initial lack of a suitable model made studies of the central nervous system (CNS) more difficult. The recognition and acceptance of the isolated nerve terminal or synaptosome as a suitable model with which to study CNS neurotransmission [3] has recently facilitated comparison between the secretion mechanisms of the peripheral and CNS.

In general, although features of transmitter release of the periphery are also apparent in the CNS [4], evidence for exocytosis in the CNS is rather limited. There is substantial ultrastructural evidence for fusion of synaptic vesicles with the synaptic plasma membrane [5, 6], but the biochemical evidence is less substantial and therefore is less convincing than the evidence for the adrenal medulla, for instance.

We have extended our work employing synaptosomes [7–9] to include not only studies of the release of noradrenaline (NA) but also of a variety of other substances, including dopamine β-hydroxylase (DA βOHase) and ATP.

Synaptosomes were prepared from cerebral cortices of male Sprague–Dawley rats as described previously [7]. The release of neurotransmitters and other substances was monitored from synaptosome layers [9, 10] where NA was measured fluorimetrically, succinate dehydrogenase (SDH) and acetylcholinesterase (AChE) manometrically, and adenosine triphosphatases (ATPases), lactate dehydrogenase (LDH) and protein colorimetrically. Dopamine β-hydroxylase was measured fluorimetrically [11]. Adenosine triphosphate was measured either by comparison of the enzymatic release of inorganic phosphate from known amounts of ATP or fluorometrically [12].

When synaptosomes were stimulated electrically (20 V, 100 Hz, 1 msec), there was a significant increase in the release of NA, adenosine triphosphate (ATP), DA βOHase and protein (Table 1). In each case the augmented release was calcium-dependent. The release of NA and ATP was dependent on the stimulation parameters employed and could be mimicked by depolarizing the preparations with K^+ (55.6 mM). In sharp contrast, on electrical stimulation there was no increment in the release of the mitochondrial marker succinate dehydrogenase (SDH), the synaptosomal membrane markers acetylcholinesterase (AChE) and sodium–potassium-activated, magnesium-dependent adenosine triphosphatase (NA^+, K^+-ATPase) or of the synaptosomal cytoplasm marker, lactate dehydrogenase (LDH).

This lack of change in the levels of these enzyme markers suggests that electrical stimulation does not simply cause increased synaptosomal breakdown. The fact that the enzymes can be measured in the incubation fluid in the absence of stimulation is perhaps indicative that a small percentage of the synaptosomes spontaneously break down at 37°. This breakdown is independent of electrical stimulation but dependent on time. Further evidence that the synaptosome is not merely 'rupturing' to release all its contents is that synaptic vesicles, as determined by the activity of anticonvulsant-sensitive Mg^{2+}-ATPase which appears to be unique to these particles [7], are not found in the incubation medium.

The release of noradrenaline is therefore more carefully controlled than 'rupturing' of synaptosomes. The question remains as to how it is brought about. Obviously it is in

Table 1. Basal and stimulated release of noradrenaline and other substances from synaptosomes*

	Basal release	Stim. release	Ratio (stim/basal)
Noradrenaline	19.2 ± 1.4	51.7 ± 2.6† nmoles . 100 $mgPr^{-1}hr^{-1}$	2.7
ATP	5.2 ± 0.8	14.6 ± 1.1† nmoles . 100 $mgPr^{-1}hr^{-1}$	2.8
DA βOHase	2.2 ± 0.5	5.5 ± 0.7† nmoles NA produced. 100 $mgPr^{-1}hr^{-1}$	2.5
Protein	15.4 ± 0.8	19.9 ± 0.8‡ ngPr released. 100 $mgPr^{-1}hr^{-1}$	1.3
LDH	2.7 ± 0.4	2.9 ± 0.3 ΔOD. 100 $mgPr^{-1}hr^{-1}$	NS§
SDH	9.2 ± 1.5	7.8 ± 2.1 μlCO_2. 100 $mgPr^{-1}hr^{-1}$	NS
AChE	10.4 ± 0.9	11.2 ± 1.5 βCO_2. 100 $mgPr^{-1}hr^{-1}$	NS
Na^+, K^+-ATPase	5.8 ± 0.6	6.2 ± 1.1 μmoles Pi. 100 $mgPr^{-1}hr^{-1}$	NS
Vesicular Mg^{2+}-ATPase	ND	ND	NS

* The release was measured as previously described [7–10] into a medium of the following composition (mM): NaCl, 153.5; $MgSO_4$, 2.8; $CaCl_2$, 2.1; KCl, 5.65; $NaHCO_3$, 1.8; sucrose, 64.3; glucose, 8.3, with the pH adjusted to 7.4. Biphasic pulses of alternating polarity (20V, 100 Hz, 1 msec) were applied to the synaptosomes when appropriate via platinum electrodes. The stimulation parameters were chosen to represent supramaximal stimulation of the synaptosomal layers. The values expressed as release per 100 mg of initial synaptosomal material are the means ± S.E.M. of six to nine experiments.

† Significant increase during electrical stimulation, $P < 0.01$.

‡ Significant increase during electrical stimulation, $P < 0.025$.

§ NS = no significant increase in ratio during electrical stimulation.

‖ ND = not detectable.

Table 2. The relative release of a variety of substances from synaptosomes under basal and stimulated conditions

	Release relative to noradrenaline*	
	Basal	Stimulated
NA	1.0	1.0
ATP	0.27 ± 0.02	0.28 ± 0.02
DA βOHase	0.11 ± 0.02	0.10 ± 0.01
Protein	0.80 ± 0.09	0.38 ± 0.06
LDH	0.14 ± 0.02	0.06 ± 0.00
SDH	0.48 ± 0.05	0.15 ± 0.01
AChE	0.54 ± 0.09	0.22 ± 0.02
Na^+, K^+-ATPase	0.30 ± 0.06	0.11 ± 0.01
Vesicular Mg^{2+}-ATPase	0	0

* The release is relative to noradrenaline basal and stimulated release shown as 1.0. Conditions are as in Table 1. The values are the means ± S.E.M. of four to six determinations rounded off to two decimal points.

close association with DA βOHase and ATP. This is seen in Table 2 where the ratios of these substances relative to NA remain constant under conditions of basal and stimulated release. These ratios for the other proteins and enzymes measure tend to decrease under conditions of electrical stimulation.

The ATP concentrations measured here do not necessarily reflect the total amount of ATP released during stimulation. In our experiments, in addition to an increment in ATP release, there is also a large increase in inorganic phosphate [13], the breakdown product of ATP, during stimulation. Experiments have shown that any exogenous ATP added to the preparations is broken down rapidly by synaptosomal ATPases and other enzymes.

These findings of rapid ATP hydrolysis are in agreement with the work of White [14], who used luciferin–luciferase to measure the ATP, but they are difficult to reconcile with our finding that there is some ATP remaining in the incubation medium after 10 min. One answer as to why some ATP remains is that perhaps it is sequestered with either NA or protein and is not accessible in this form to the action of ATPases. However, the important feature remains that during electrical stimulation, the concentration of ATP in the medium is increased more than 2-fold.

It is unlikely that the increase in overall protein in Table 1 can be accounted for by increased DA βOHase levels alone. One of many possibilities as to the origin of the remainder of the protein is that at least in part it could be chromogranin, found at other sites of NA release [15].

None of the evidence presented here is inconsistent with the involvement of exocytosis in the release of NA from synaptosomes. The process is calcium-dependent; the organelles of the synaptosome are not released, as shown by the use of enzyme markers for synaptosomal cytoplasm, mitochondria and synaptic vesicle. There is, however, an augmented release of NA, ATP and DA βOHase, presumably from synaptic vesicles [16, 17], upon stimulation. The release of each of these is intimately associated with that of the others as the release ratios remain relatively constant for basal and stimulated release. The ATP released is in a form apparently inaccessible to enzymatic destruction, and perhaps bound to NA, DA βOHase and even chromogranin in a suitable form for traversing the synaptic cleft.

Biochemical Pharmacology Section,
Wyeth Institute of Medical Research,
Huntercombe Lane South,
Taplow,
Maidenhead, Berkshire,
U.K.
MICHAEL G. WYLLIE

Pharmacology Section,
Department of Pharmacology,
Heriot-Watt University,
79 Grassmarket,
Edinburgh,
U.K.
JOHN C. GILBERT

REFERENCES

1. W. S. Douglas, *Br. J. Pharmac.* **34**, 451 (1968).
2. L. B. Geffen, B. G. Livett and R. A. Rush, *J. Physiol., Lond.* **204**, 58p (1969).
3. J. S. De Belleroche and H. F. Bradford, in *Progress in Neurobiology* (Ed. G. A. Kerkut and J. W. Phillis), Vol. 1, pp. 275–298. Pergamon Press, Oxford (1975).
4. C. W. Cotman, J. W. Haycock and R. F. White, *J. Physiol., Lond.* **254**, 475 (1976).
5. P. R. C. Howe, J. A. P. Rostas, E. M. Fenwick and B. G. Livett, *Proc. Aust. biochem.Soc.* **8**, 95p (1975).
6. R. C. Fried and M. P. Blaustein, *J. Cell Biol.* **78**, 685 (1978).
7. J. C. Gilbert and M. G. Wyllie, *Br. J. Pharmac.* **56**, 49 (1976).
8. J. C. Gilbert, J. M. Allen, B. G. Townsend and M. G. Wyllie, *Neuropharmacology* **17**, 419 (1978).
9. J. C. Gilbert, D. V. Davison and M. G. Wyllie, *Neuropharmacology* **17**, 417 (1978).
10. J. C. Gilbert and M. G. Wyllie, *Br. J. Pharmac.*, in press.
11. A. D. Smith and N. Kirshner, *Molec. Pharmac.* **3**, 52 (1967).
12. J. R. Williamson and B. Corkey, in *Methods in Enzymology* (Ed. J. Lowenstein), Vol. 13, pp. 434–472. Academic Press, New York (1969).
13. J. C. Gilbert and M. C. Wyllie, *Br. J. Pharmac.* **60**, 312p (1977).
14. T. D. White, *J. Neurochem.* **30**, 329 (1978).
15. R. J. Baldessarini, in *Handbook of Psychopharmacology* (Eds. L. L. Iversen, S. D. Iversen and S. H. Snyder), Vol. 3, *Biochemistry of Biogenic Amines*, pp. 37–137. Plenum Press, New York (1975).
16. M. Bisby, M. Fillenz and A. Smith, *J. Neurochem.* **20**, 245 (1973).
17. A. Wada, T. Miyashita, F. Izumi, T. Kashimoto and M. Oka, *Biochem. Pharmac.* **26**, 986 (1977).