Receptor modulation in brain slices

E.W. Karbon and S.J. Enna

Brain tissue preparations provide a window on the regulation of neuroreceptor function that cell-free preparations cannot offer. The view reveals complex neuromodulator interactions.

MANY neurotransmitters influence neuronal function by directly affecting either of two important signal transduction mechanisms. Of these mechanisms, the most well-characterized, is the receptor-coupled adenylate cyclase system[1]. Stimulation of the cyclase enzyme enhances the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), while inhibition decreases cAMP production. The cyclic nucleotide then acts as a "second messenger" by stimulating a protein kinase that phosphorylates selected proteins.

The other important receptor-coupled effector system is phosphatidylinositol turnover[2]. In response to receptor activation, phospholipase C degrades phosphatidylinositol 4,5-bisphosphate, a phospholipid, to dual second messengers. One messenger, inositol triphosphate, liberates calcium ions from internal stores; the other, diacylglycerol, stimulates protein kinase C that, like the cAMP-dependent enzyme, phosphorylates multiple protein substrates.

The ability of neurotransmitters to influence these processes has routinely been examined in brain slices, intact tissue preparations that closely approximate *in vivo* conditions. These slices are maintained in an aerated, physiological buffer and are pre-labelled with a substrate precursor such as ^{3}H-adenine, which is converted to ^{3}H-ATP, or ^{3}H-inositol, which is incorporated into phospholipid pools. Enzyme activity in response to receptor activation can then be assessed by measuring the levels of radiolabelled product that have accumulated[3].

Mixed reception

Given the importance of identifying the events which follow receptor activation, the brain slice preparation is a valuable tool. Recent studies have shown that, although the direct stimulation of second messenger formation by neurotransmitters is often sufficient to account for the biochemical effects they provoke, many neurotransmitter receptors are also subject to the regulatory influences of compounds acting at distinct receptor sites. Such compounds are called "neuromodulators" since they indirectly modify the biochemical response resulting from activation of another receptor.

For example, brain slices exposed to noradrenaline, which stimulates both α- and β-adrenergic receptors, show a greater accumulation of cyclic AMP than those treated with isoprenaline, a selective β-adrenergic agonist[4,5] (Table 1). These results suggest that α-adrenergic receptors, like the β-adrenergic components, may be directly coupled to adenylate cyclase. But α-agonists such as 6-fluoronoradrenaline (6-FNA) do not of themselves stimulate cAMP accumulation (Table 1).

Table 1 Neuromodulator effects on cAMP accumulation

	cAMP accumulation (% conversion)	
	Basal	Isoprenaline
No addition	0.06	0.38
6-FNA (10 μM)	0.07	0.77
Baclofen (50 μM)	0.11	1.14
PDBu (10 μM)	0.06	1.20
Noradrenaline (100 μM)	0.80	—

The influence of various agents on cAMP accumulation in rat brain cerebral cortical slices. Results are expressed as per cent conversion, which represents the percentage of total tritium present as ^{3}H-cyclic AMP. 6-FNA, 6-fluoronoradrenaline; baclofen, β-p-chlorophenyl γ-aminobutyric acid; PDBu, phorbol 12, 13-dibutyrate. (Adapted from refs 3,7,9.)

When, however, rat brain slices are exposed to both 6-FNA and isoprenaline, the cAMP response equals that obtained when noradrenaline alone is used. These findings can be taken as evidence that α-adrenergic receptors are indirectly coupled to the adenylate cyclase system and that noradrenaline, through interaction with these sites, can function as a neuromodulator.

Similar results have recently emerged from studies using the agonist β-p-chlorophenyl γ-aminobutyric acid (baclofen). Baclofen specifically recognizes a γ-aminobutyric acid (GABA) receptor subtype which is referred to as the $GABA_B$ site[6]. Like 6-FNA, baclofen augments cAMP accumulation markedly in brain slices following exposure to a number of β-adrenergic receptor stimulants, while being ineffective alone in this regard[7] (Table 1). The augmenting response elicited by both $GABA_B$ and α-adrenergic agonists is independent of phosphodiesterase activity and totally dependent upon the presence of extracellular calcium, suggesting a common mechanism of action[3,8].

The ability to augment cyclic AMP formation is not limited to endogenous substances, for it can be elicited by phorbol esters, compounds that mimic the action of diacylglycerol and activate protein kinase C[9,10] (Table 1). This finding is intriguing in that it suggests that activation of phospholipase C and the subsequent generation of diacylglycerol may be the mechanism responsible for the augmentation observed in response to $GABA_B$ and α-adrenergic receptor activation. Such a mechanism would point to a positive interaction between two major signalling pathways[11].

A cut above

Brain slice preparations have been invaluable in elucidating a role for α-adrenergic and $GABA_B$ receptors, because the augmentation phenomenon is not observed in cell-free preparations. The reason for this absence is not obvious, but may be related to the fact that direct receptor-mediated stimulation of adenylate cyclase is also difficult to detect in membrane preparations. Alternatively, a requirement for some soluble factor may preclude the detection of any augmentation in isolated plasma membranes.

Since the augmentation response is a functional measure of receptor activity, this system can be used to screen for potential α-adrenergic and $GABA_B$ receptor agonists and antagonists, which cannot be identified with receptor binding analysis. Brain slice preparations can also disclose the biochemical consequences of agents like phorbol esters that act at intracellular sites. And while the physiological relevance of receptor-mediated cAMP augmentation is still unknown, brain slice studies have hinted that the pharmacological manipulation of neuromodulator receptors might hold considerable therapeutic potential. □

William Karbon is at the Department of Pharmacology, Yale University School of Medicine, PO Box 3333, New Haven, Connecticut 06510, USA. Salvatore Enna is research director at Nova Pharmaceutical Corp, 5210 Eastern Avenue, Baltimore, Maryland 21224, USA.

1. Ross, E.M. & Gilman, A.G. *A. Rev. Biochem.* **49**, 553 – 564 (1980).
2. Berridge, M.J. *Biochem. J.* **220**, 2625 – 2628 (1984).
3. Duman, R.S. *et al. J. Neurochem.* **47**, 800 – 810 (1986).
4. Perkins, J.P. & Moore, M.M. *J. Pharmac. exp. Ther.* **185**, 371 – 378 (1973).
5. Pilc, A. & Enna, S.J. *J. Pharmac. exp. Ther.* **237**, 725 – 730 (1986).
6. Enna, S.J. & Karbon, E.W. in *Benzodiazepine/GABA Receptors and Chloride Channels* (eds Olsen, R.W. & Venter, J.C.) 41 – 56 (Liss, New York, 1986).
7. Karbon, E.W. & Enna, S.J. *Molec. Pharmacol.* **27**, 53 – 59 (1985).
8. Schwabe, U. & Daly, J.W. *J. Pharmac. exp. Ther.* **202**, 134 – 143 (1977).
9. Karbon, E.W. *et al. J. Neurochem.* (in the press).
10. Castagna, M. *et al. J. biol. Chem.* **257**, 7847 – 7851 (1982).
11. Enna, S.J. & Karbon, E.W. *Trends pharmac. Sci.* (in the press).