Biochemical Pharmacology, Vol. 23, pp. 1927–1935. Pergamon Press, 1974. Printed in Great Britain.

COMMENTARY

UPTAKE MECHANISMS FOR NEUROTRANSMITTER AMINES

L. L. IVERSEN

MRC Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD, England

SPECIALIZED uptake mechanisms exist in various types of neurone to mediate the transport of neurotransmitter amines from the external medium. Such mechanisms are known to exist in noradrenergic neurones in the peripheral sympathetic nervous system and in the CNS, in dopaminergic and tryptaminergic neurones in CNS, and in cholinergic neurones in the periphery and CNS. In cholinergic neurones, however, the transport mechanism is for the transmitter precursor choline rather than for acetylcholine. The detailed biochemical and pharmacological properties of these amine transport mechanisms have been reviewed recently elsewhere.[1–4] Similar mechanisms are thought to exist in neurones that use the amino acids GABA, glycine and glutamate as neurotransmitters,[5,6] but these will not be discussed here. The present review will concentrate on some of the neuropharmacological implications of our present knowledge of transmitter uptake systems, suggest how this information may be used for research purposes, and point out some of the gaps in our understanding.

NORADRENALINE

Neuronal and extraneuronal uptake mechanisms. The most extensively studied uptake systems are those responsible for the accumulation of noradrenaline (NA) by adrenergic neurones, and in various peripheral effector tissues. The neuronal uptake of noradrenaline is mediated by a high affinity transport system (K_m = 0·2–0·4 μM) located in the axonal membrane of adrenergic neurones.[1,2] This system is highly dependent on the presence of sodium ions in the external medium and is stereochemically selective in most species and tissues for the naturally occurring (−)-isomer of NA. The uptake sites are not completely specific for NA and a variety of other catecholamines and β-phenylethylamines can act as alternative substrates and are selectively accumulated in adrenergic neurones. The neuronal uptake of NA is potently inhibited by cocaine, by various sympathomimetic amines and by tricyclic antidepressant drugs of the imipramine and amitriptyline series. Among the latter drugs, the *N*-desmethyl derivative desipramine remains one of the most potent inhibitors of NA uptake known, with a K_i of approximately 0·01–0·05 μM.

In addition to the neuronal uptake of NA, sometimes known as Uptake$_1$, there is also a second uptake system present in various smooth muscle and glandular tissues known as Uptake$_2$.[2,7] This is again a membrane transport system, but differs

from $Uptake_1$ in having a very much lower affinity for NA (K_m = approximately 250 μM). It is also less markedly sodium-dependent and shows no stereochemical selectivity for (−) and (+)-NA. *N*-substituted catecholamines such as adrenaline and isoprenaline are taken up with higher affinity than NA by $Uptake_2$ sites. Although the extraneuronal uptake sites have low affinity for catecholamines they are far more numerous in most peripheral organs than the neuronal uptake sites, so that the rates of removal of catecholamines from the total volume of the extracellular space by the $Uptake_1$ and $Uptake_2$ mechanisms may in fact be comparable despite the low affinity of the $Uptake_2$ sites for the amine substrates. Catecholamines taken up by $Uptake_2$ sites are normally rapidly degraded by the enzymes monoamine oxidase and catechol-*O*-methyl transferase in smooth muscle or other effector tissues. If, however, tissues are exposed to very high catecholamine concentrations the rate of extraneuronal uptake may exceed the metabolic capacity of the tissue, and unchanged amine will then accumulate in the extraneuronal sites.[8] This led to the original discovery of the extraneuronal uptake processes, since accumulations of unchanged catecholamines in extraneuronal sites can be readily visualized by fluorescence histochemical methods or measured biochemically. $Uptake_2$ is potently inhibited by *O*-methylated catecholamine metabolites such as metanephrine, by various haloalkylamines such as phenoxybenzamine and by several steroids such as β-oestradiol, corticosterone, testosterone and (less potently) by cholesterol.[9,10]

Role of uptake in inactivation of NA. The neuronal recapture of NA after its release from adrenergic nerve terminal is now well established as an important mechanism in the inactivation of neurally released NA. The exact extent of this recapture, however, is not clear. In organs with a dense sympathetic terminal innervation, in which neuronal release sites are situated relatively close to the transmitter target cells in the effector tissue, the recapture mechanism seems likely to be far more efficient than in tissues, such as arterial smooth muscle, in which there is only a sparse sympathetic innervation and the transmitter substance has to penetrate for a relatively long distance from the nerve terminals to the sites of actions. The available evidence concerning the extent of neuronal recapture of released NA has come almost entirely from densely innervated organs such as the nictitating membrane, cardiac tissues and vas deferens. In such tissues the overflow of NA and NA metabolites in response to stimulation of the sympathetic innervation increases by 3–4 fold when the tissues are exposed to cocaine.[11,12] This suggests that some 70–80 per cent of the released NA is normally removed by the neuronal recapture mechanism. It is now recognised that the much larger increases in NA overflow evoked by treatment with phenoxybenzamine in such experiments are only partially attributable to an inhibition of NA uptake by this drug. Several recent studies suggest that phenoxybenzamine and other α-adrenoceptor antagonists increase the overflow of NA in response to sympathetic nerve stimulation by blocking a negative feedback mechanism in adrenergic nerve terminals whereby released NA acts upon presynaptic α-adrenoceptors to inhibit further transmitter release.[13,14] In addition to such an action phenoxybenzamine is also a potent inhibitor of the $Uptake_2$ mechanism and thus prevents released NA from being taken up and metabolized in the postsynaptic tissues, an action which again tends to increase the amount of unchanged NA appearing in overflow experiments. From studies on the overflow of NA and its metabolites from stimulated tissues after exposure to inhibitors of $Uptake_1$ and/or $Uptake_2$ it appears that these

two mechanisms constitute alternative routes for terminating the actions of neurally released NA. If $Uptake_1$ sites are blocked with cocaine a larger proportion of the released NA is diverted to the "$Uptake_2$-followed-by-metabolism" mechanism.[12] This mode of inactivation also becomes more important than neuronal recapture under conditions in which the sympathetic nerves are stimulated at frequencies of 10 Hz or more; this appears to lead to a situation in which the release of NA occurs more rapidly than can be dealt with by the $Uptake_1$ mechanism. Thus, cocaine causes only a small increase in NA overflow if stimulation is carried out at 25 Hz,[11] and $Uptake_1$ inhibitors cause only small potentiation of the responses of sympathetically innervated tissues to nerve stimulation at high frequencies, although such potentiation may be marked when tissues are stimulated at more physiologically normal frequencies (around 1 Hz). This balance between the $Uptake_1$ and $Uptake_2$ mechanisms could be of importance since it implies that $Uptake_2$ offers an alternative mechanism for transmitter inactivation under conditions in which neuronal recapture is inadequate. Such conditions might occur in densely innervated tissues when the sympathetic innervation is fired at high frequencies, or it might be important in less densely innervated tissues even at lower stimulation frequencies, since in such tissues the $Uptake_2$ sites are far more numerous than the neuronal recapture sites, by comparison with densely innervated organs. The interaction between the tissue uptake mechanisms, which catalyse a rapid removal of NA from the extracellular space, and the negative feedback mechanisms involved in controlling transmitter release from presynaptic terminals remains to be explored. The latter mechanisms involve either an interaction of NA with presynaptic α-adrenoceptors as described above, or a stimulation of prostaglandin release by NA.[15] These control mechanisms will only be activated under conditions in which NA accumulates in the extracellular space after its release, for example when sympathetic nerves are stimulated continuously at relatively high frequencies. Such mechanisms could not, of course, operate if the removal of released NA by $Uptake_1$ and/or $Uptake_2$ occurred very rapidly, since NA would then never accumulate in the extracellular space.

It is likely that $Uptake_2$ sites are important not merely as a back-up mechanism to $Uptake_1$ in disposing of neurally released NA, but also as a mechanism for the rapid inactivation of catecholamines released into the circulation from the adrenal medulla. Adrenaline, for example, is taken up by $Uptake_2$ sites with greater affinity than NA. $Uptake_2$ sites are present in abundance in the smooth muscle cells of small blood vessels, and are thus ideally situated to remove circulating catecholamines during their passage through peripheral vascular beds. $Uptake_2$ sites also occur in several smooth muscle tissues that are not innervated by sympathetic nerves,[16] suggesting that this uptake mechanism plays some role not necessarily associated with adrenergic neurotransmission. There is, however, little direct evidence to support this hypothesis. If it were correct one would expect that substances known to act as specific inhibitors of $Uptake_2$, such as steroids, *O*-methylated catecholamines and certain haloalkylamines should prolong and potentiate the actions of circulating catecholamines more effectively than inhibitors of $Uptake_1$ such as cocaine or desipramine. The possible effects of circulating or tissue concentrations of steroids such as cholesterol in regulating the activity of $Uptake_2$ sites could also have important physiological implications. If "$Uptake_2$-followed-by-metabolism" is an important mechanism for inactivating circulating catecholamines then an excess of cholesterol would be

expected to potentiate physiological responses to adrenal catecholamines by rendering cardiovascular tissues more responsive to such amines.

Pharmacological implications of NA uptake. The existence of NA uptake systems in adrenergic neurones and in effector tissues has many implications for understanding drugs actions on adrenergic mechanisms. It is probable, for example, that the effects of drugs such as cocaine or desipramine in potentiating the responses of effector tissues to neurally released or administered catecholamines can be explained almost entirely by virtue of the inhibition of neuronal uptake sites caused by these drugs. This potentiation of effector responses by uptake inhibitors also implies that the neuronal uptake mechanism is capable of removing catecholamines from the biophase sufficiently rapidly to limit the concentration of amine in the extracellular space adjacent to adrenoceptor sites in the effector tissues. Since this potentiation occurs both for NA released from adrenergic terminals and for externally applied catecholamines this means that neuronal uptake from the biophase adjacent to such receptors can occur more rapidly than the rate of penetration of applied amine from the external medium. This has further important implications in assessing agonist responses in sympathetically innervated tissues. If a series of agonist drugs is compared a quite misleading set of results regarding their relative potencies may be obtained unless some precaution is taken to inactivate the neuronal uptake sites. Different agonist amines will also differ in the relative importance of the neuronal uptake mechanism in limiting their concentration at adrenoceptor sites. This effect will be most important for amines which have both a high affinity for uptake sites and are potent agonists. Such amines will stimulate receptor responses at concentrations in which uptake sites are not saturated by the amine and are thus operating most effectively. Weak agonists, on the other hand, will generally need to be used at concentrations which are so high that neuronal uptake sites will be completely saturated and thus relatively ineffective. This explains, for example, why the responses of effector tissues to the (−) isomers of catecholamines are potentiated to a much greater extent by the removal of uptake sites following sympathetic denervation or after cocaine treatment than the less potent (+)-isomers, even though both (−) and (+)-isomers are substrates for the neuronal uptake mechanism.[17] Other amines such as isoprenaline are not substrates at all for the neuronal uptake sites, and their effects are thus not affected by removal or inhibition of such uptake sites. Responses to isoprenaline, however, can still be potentiated by inhibition of $Uptake_2$ sites.[18] The affinity of agonists for both $Uptake_1$ and $Uptake_2$ sites thus needs to be considered in assessing effector tissue responses. The ideal situation for measuring such responses might be to use a sympathetically denervated tissue treated with a selective $Uptake_2$ inhibitor such as corticosterone.

Apart from considering the role of uptake mechanisms in limiting the concentrations of agonist drugs available at adrenoceptor sites, uptake can also be important in explaining the pharmacological actions of drugs that depend on a selective accumulation in adrenergic nerve endings in order to exert their actions. Several β-phenethylamines, for example, metaraminol and α-methyl NA, can be selectively accumulated in adrenergic nerves where they replace NA and are stored and released as "false transmitters". The high affinity that such amines have for neuronal uptake sites, together with their resistance to metabolic degradation probably accounts for their long half lives in adrenergic nerves; uptake probably accounts not only for their

initial entry but also for their conservation through multiple cycles of release and recapture. Other sympathomimetic amines that act indirectly by displacing NA from adrenergic nerve terminals may also depend upon the neuronal uptake mechanism to enter the adrenergic terminals. Tyramine, for example, is a substrate for the neuronal uptake mechanism and its actions are antagonized by drugs such as cocaine that block such uptake sites. Other indirectly acting amines, however, such as amphetamine may be sufficiently lipid soluble to achieve an adequate penetration of adrenergic terminals without requiring the mediation of uptake mechanisms. Adrenergic neurone blocking drugs such as bretylium and guanethidine are also selectively concentrated by uptake in adrenergic nerves and this, together with their local anaesthetic properties, may account for their selective actions in blocking nerve conduction in adrenergic terminal fibres. Another drug which owes the selectivity of its actions on adrenergic neurones to its ability to act as a substrate for neuronal uptake is 6-hydroxydopamine. The selective actions of this neurotoxic substance on adrenergic nerves derive from the selective accumulation of this amine by neuronal uptake sites in adrenergic neurones.

DOPAMINE

Studies of the uptake of tritiated dopamine by slices of homogenates of the dopamine-rich neostriatum of mammalian brain have demonstrated that dopaminergic neurones possess their own uptake mechanism.[1–3] This is similar in many respects to the neuronal uptake mechanism for NA; dopamine uptake sites are sodium-dependent and have a high affinity for dopamine (K_m = 0·4 μM). NA is also taken up by the dopaminergic neurones, but with a lower affinity (K_m = 2·0 μM) and with no stereochemical selectivity. Other β-phenylethylamines can inhibit dopamine uptake, and the structure-activity relationships for dopamine uptake sites seem to be similar to those previously described for NA uptake sites in noradrenergic neurones. It was at first thought that the (+) and (−)-isomers of amphetamine were equipotent as inhibitors at dopamine uptake sites, but subsequent findings indicate that, as in noradrenergic neurones, (+)-amphetamine is considerably more potent than (−)-amphetamine. The most striking difference in inhibitor sensitivity is the relative ineffectiveness of tricyclic antidepressant drugs as inhibitors of dopamine uptake. Desipramine, for example, is approximately one thousand times less potent as an inhibitor of dopamine uptake than of NA uptake.[19] On the other hand, dopamine uptake sites are potently inhibited by benztropine and certain related anticholinergic drugs. Benztropine is approximately twenty times more potent as an inhibitor of dopamine uptake than of NA uptake.[19] These findings make it clear that the uptake sites in dopaminergic neurones are different from those in noradrenergic neurones. The functional importance of dopamine uptake, however, remains obscure, although by analogy with the situation in noradrenergic neurones a role in transmitter inactivation seems most probable. The pharmacological importance of dopamine uptake is also unclear. It is possible that benztropine and related anticholinergic drugs used in the treatment of Parkinsonism may owe some of their actions to an inhibition of dopamine uptake, thus potentiating and prolonging the actions of any residual dopamine released in the Parkinsonian brain. Such drugs, however, do not appear to produce signs of dopaminergic hyperactivity when administered to normal animals.

5-HYDROXYTRYPTAMINE

5-hydroxytryptamine (5-HT) is also taken up by a specific high affinity mechanism that appears to be associated with tryptaminergic (5-HT containing) neurones in mammalian brain.[3,4] The uptake of labelled 5-HT by synaptosomes is greatly reduced after surgical lesions of the raphe nuclei in rat brain, which cause a selective degeneration of 5-HT containing nerve terminals.[4] Autoradiographic studies indicate that only a very small proportion of the synaptosomes in rat forebrain homogenates are capable of 5-HT uptake (less than 0·067 per cent), presumably corresponding to the relatively small number of tryptaminergic synaptic terminals.[4] 5-HT uptake is again sodium-dependent and is mediated by a high affinity mechanism (K_m = 0·2 μM). It is inhibited by various indolamines, but in general not potently inhibited by β-phenylethylamines. 5-HT is also taken up, although with considerably lower affinity (K_m = 8 μM) by both dopaminergic and noradrenergic neuronal uptake sites. This makes studies of 5-HT uptake difficult unless very low concentrations (*ca.* 0·1 μM) of amine are used, since otherwise 5-HT uptake occurs into both tryptaminergic and catecholamine uptake sites. It has recently been found that the high affinity uptake of 5-HT in tryptaminergic neurones is very potently inhibited by tricyclic antidepressant drugs of both the imipramine and amitriptyline series, although the structure–activity relations among such drugs as inhibitors of 5-HT uptake are different from those for inhibition of NA uptake by these compounds. For example, the tertiary amine derivatives imipramine and amitriptyline are more potent inhibitors of 5-HT uptake than their desmethyl derivatives desipramine and nortriptyline, a situation which is the converse of that for inhibition of NA uptake. 3-chloroimipramine is the most potent inhibitor of 5-HT uptake sites so far described (K_i approx. 0·1 μM). The inhibition of 5-HT uptake sites in brain by tricyclic antidepressants and by indolamines closely parallels the behaviour of 5-HT uptake sites in blood platelets.[20] Indeed, the parallels are so close as to suggest that the 5-HT uptake mechanisms in platelets and in tryptaminergic neurones may be identical. The finding that antidepressant drugs are powerful inhibitors of both NA and 5-HT uptake sites in brain leaves some confusion as to which of these effects is critical for the antidepressant action of these drugs—if indeed this action does depend upon amine uptake inhibition. This question, however, is difficult to answer with the drugs presently available, since no tricyclic antidepressant drugs have been described which are selective inhibitors of either NA or 5-HT uptake. No doubt such compounds would not be difficult to discover by careful screening, and investigations of the clinical actions of such selective amine uptake inhibitors in affective disorders might be most interesting.

The functional importance of 5-HT uptake is unknown, as with the dopamine uptake process. Pharmacologically the existence of 5-HT uptake sites almost certainly underlies the selective neurotoxic actions of the compound 5,6-dihydroxytryptamine (5,6-HT) on 5-HT containing neurones.[21] 5,6-HT has a considerably higher affinity for 5-HT uptake sites than for NA or dopamine uptake sites, and this implies that it is selectively concentrated in 5-HT containing neurones in which it acts in a manner similar to 6-hydroxydopamine in adrenergic neurones.

CHOLINE

The enzyme acetylcholinesterase is present at all cholinergic synapses and is responsible for the rapid inactivation of released acetylcholine by enzymic hydrolysis.

It might thus appear that cholinergic neurones have no need of a high affinity amine uptake mechanism, but recent findings indicate that this is not in fact the case. It has been known for some time that cholinergic neurones are highly dependent upon a continuing external supply of the precursor choline in order to maintain the synthesis of acetylcholine. It has been assumed that such neurones obtained their supply of choline by an uptake of choline by a relatively low affinity transport system (K_m approx. 200 μM) that had been described in many tissues. Choline is, of course, required by many cells other than cholinergic neurones as a precursor for the biosynthesis of phospholipids. More recently, however, a high affinity uptake system for choline has been described which appears to be specifically located in cholinergic neurones.[6,22] Studies of the uptake of labelled choline by homogenates of rat brain revealed the presence of two components on kinetic analysis. A low affinity component (K_m = 100 μM) was present in all brain regions. However, at very low external choline concentrations a second component with much higher affinity (K_m 1–3 μM) predominated. This component, unlike the low affinity system, was not present in brain regions which contain only few cholinergic neurones, such as cerebellum, but was prominent in acetylcholine-rich regions such as the striatum.[22] The high affinity uptake component was also markedly reduced in homogenates of rat hippocampus following surgical lesions that destroyed the cholinergic input to this brain region, suggesting that high affinity choline uptake sites were specifically associated with cholinergic nerve terminals.[4] This was also the case in guinea pig ileum, in which high affinity choline uptake sites occurred only in those parts of the organ known to contain cholinergic fibres.[6] Further evidence that the high affinity uptake sites for choline are associated with cholinergic neurones in both ileum and brain came from the finding that in each case ^{3}H-choline taken up from low external concentrations by this mechanism was rapidly and completely converted into ^{3}H-acetylcholine, whereas a much smaller proportion of the material taken up from higher choline concentrations was thus converted. A high affinity uptake of choline has been described in studies of choline uptake by synaptosomes prepared from squid CNS.[23]

The high affinity uptake of choline by cholinergic nerves probably represents an important mechanism for recapturing this essential transmitter precursor substance after acetylcholine has been released from cholinergic nerve terminals and enzymically hydrolysed in the synaptic cleft. This may be particularly important for cholinergic neurones in the CNS, since brain tissue is incapable of synthesising choline *de novo*.

The potent inhibition of choline uptake sites by hemicholinium-3 (K_i = approx. 1 μM) also explains the inhibitory effects of this drug on acetylcholine synthesis in cholinergic neurones. The finding that acetylcholine has an appreciable affinity for choline uptake sites may also account for the remarkable capacity of brain tissue to accumulate this amine when acetylcholinesterase has been inactivated with organophosphorus inhibitors.

GENERAL CONCLUSIONS

The various amine uptake mechanisms associated with adrenergic, tryptaminergic and cholinergic neurones share many common features. In each case uptake is mediated by a transport mechanism which is completely dependent upon the presence of sodium ions in the external medium, and which has a very high affinity for

the substrate amine, with K_m values in the range 0·2–1·0 μM. These high affinity uptake processes are capable of generating extremely high concentration ratios between the external medium and the intraneuronal space. Such concentrative capacities are further enhanced by the fact that amines accumulated by the high affinity uptakes do not remain free in the axoplasm but are rapidly sequestered in intraneuronal amine storage vesicles, or in the case of choline rapidly converted into acetylcholine.

The knowledge of the existence of such mechanisms can be exploited in many different ways for research purposes. The presence of specific amine uptake sites in brain or in peripheral tissues can be used, for example, as a sensitive indicator of the presence and density of innervation by various transmitter-specific neurones. Since uptake sites disappear almost completely after surgical lesions that lead to terminal degeneration in adrenergic, tryptaminergic or cholinergic pathways the presence of uptake sites can be used to indicate the extent of terminal degeneration of various pathways in CNS following such lesions. The development of simple techniques for measuring the rate of uptake of radioactively labelled amines by synaptosomes in homogenates of brain incubated *in vitro* has greatly facilitated such studies.[3,4] The ability to introduce radioactively labelled amines selectively into NA, dopamine, 5-HT or acetylcholine-containing neurones by means of the uptake mechanisms also offers many possibilities for studies of transmitter metabolism, storage, release and inactivation in complex neuronal tissues such as the brain. The selective labelling of transmitter-specific neurones can also be used in conjunction with radioautographic techniques for the identification and mapping of aminergic pathways,[24,25] although this cannot at present be applied easily to cholinergic neurones since neither choline nor acetylcholine can be retained in tissue specimens after conventional fixation procedures. Radioautographic procedures may have advantages over other histochemical and cytochemical techniques for localizing the adrenergic or tryptaminergic neurones, particularly at the fine structural level. Autoradiographic localization of tritium-labelled catecholamines or 5-HT can be performed readily at the electron microscope level, where other cytochemical techniques are at present difficult to apply, especially to adrenergic neurones in the CNS. The possible ambiguities that may result by a non-selective uptake of tritiated NA by dopaminergic neurones, or by 5-HT uptake into adrenergic neurones can be avoided if careful attention is paid to the concentration of labelled amine used, and the possible use of labelled amines in conjunction with selective inhibitors of one or other amine uptake.

The functional importance of dopamine and 5-HT uptake is still unclear. If, however, these mechanisms are involved in terminating the actions of dopamine and 5-HT after their neural release, the pharmacological considerations discussed in detail above for NA uptake should be expected to hold for these amines also. In particular one would expect that inhibitors of these amine uptake sites will prolong and potentiate the actions of dopamine and 5-HT in CNS, and that the presence of such uptake sites will affect the availability of various agonist drugs at dopamine and 5-HT receptor sites in CNS.

The loss of high affinity amine uptake sites following denervation, both in the periphery and in the CNS, also has an important bearing on the phenomenon of denervation supersensitivity in different transmitter specific pathways. A loss of uptake

sites following sympathetic denervation of peripheral organs is known to play an important role in the immediate development of supersensitivity in such organs, and it is likely that similar phenomena also occur in the CNS and that they apply to both dopaminergic and tryptaminergic pathways as well as to noradrenergic ones.

Our present knowledge of the biochemical and pharmacological properties of amine uptake mechanisms should be sufficient to serve as a useful guide for the future development of more selective inhibitors of the various different uptake processes. Many of the inhibitors that are currently available suffer from being inadequately specific in that they will affect more than one of the amine uptake systems. If more specific inhibitors were available they could serve as valuable tools for further studies of the functional and pharmacological importance of individual amine uptake mechanisms, and such drugs might—in the case of NA or 5-HT uptake— also have clinical applications as more selective antidepressant agents.

REFERENCES

1. L. L. IVERSEN, *Br. J. Pharmac.* **41,** 571 (1971).
2. L. L. IVERSEN, *Br. Med. Bull.* **29,** 130 (1973).
3. S. H. SNYDER, M. J. KUHAR, A. I. GREEN, J. T. COYLE and E. G. SHASKAN, *Int. rev. Neurobiol.* **13,** 127 (1970).
4. M. J. KUHAR, *Life Sci.* **13,** 1623 (1973).
5. L. L. IVERSEN, J. S. KELLY, M. MINCHIN, F. SCHON and S. R. SNODGRASS, *Brain Res.* **62,** 567 (1973).
6. S. H. SNYDER, H. A. YAMAURA, C. B. PERT, W. L. LOGAN and J. P. BENNETT, in *New Concepts in Neurotransmitter Regulation* (Ed. A. J. MANDELL), p. 195. Plenum Press, New York (1973).
7. J. S. GILLESPIE, *Br. Med. Bull.* **29,** 136 (1973).
8. S. L. LIGHTMAN and L. L. IVERSEN, *Br. J. Pharmac.* **37,** 638 (1969).
9. P. J. SALT, *Eur. J. Pharmac.* **20,** 329 (1972).
10. P. J. SALT and L. L. IVERSEN, *Nature New Biol.* **238,** 91 (1972).
11. S. Z. LANGER, *J. Physiol. (Lond.)* **208,** 515 (1970).
12. J. HUGHES, *Br. J. Pharmac.* **44,** 472 (1972).
13. M. A. ENERO, S. Z. LANGER, R. P. ROTHLIN and F. J. E. STEFANO, *Br. J. Pharmac.* **44,** 672 (1972).
14. K. STARKE, *N.S. Archiv. Pharmac.* **274,** 18 (1972).
15. E. W. HORTON, *Br. Med. Bull.* **29,** 148 (1973).
16. G. BURNSTOCK, J. R. MCLEAN and M. WRIGHT, *Br. J. Pharmac.* **43,** 180 (1971).
17. S. Z. LANGER and U. TRENDELENBURG, *J. Pharmac. exp. Ther.* **167,** 117 (1969).
18. R. W. FOSTER, *Br. J. Pharmac.* **31,** 466 (1967).
19. A. S. HORN, J. T. COYLE and S. H. SNYDER, *Mol. Pharmac.* **7,** 66 (1971).
20. J. M. SNEDDON, in *Progress in Neurobiology* (Eds. G. A. KERKUT and J. W. PHILLIS), Vol. 1, p. 151. Pergamon Press, Oxford (1973).
21. H. G. BAUMGARTEN, A. BJORKLUND, L. LACHENMAYER, A. NOBIN and U. STENEVI, *Acta physiol. scand.* suppl. 373.
22. H. J. YAMAMURA and S. H. SNYDER, *J. Neurochem.* **21,** 1355 (1973).
23. V. P. WHITTAKER, M. J. DOWDALL and A. F. BOYNE, *Biochemical Society Symposia* No. 36, p. 49 (1972).
24. L. L. IVERSEN and F. E. SCHON, in *New Concepts in Neurotransmitter Regulation* (Ed. A. J. MANDELL), p. 153. Plenum Press, New York (1973).
25. T. HOKFELT and A. LJUNGDAHL, *Prog. Brain Res.* **34,** 87 (1971).