Br. J. Pharmac. (1971), **41**, 571–591.

Role of transmitter uptake mechanisms in synaptic neurotransmission

Third Gaddum Memorial Lecture: Royal College of Surgeons, September 1970

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Introduction

I am deeply grateful to the Trustees of the Gaddum Memorial Fund for inviting me to deliver the Third Gaddum Memorial Lecture. I feel singularly ill-equipped to do justice to the honourable task thus conferred on me, particularly in view of the very high standards set by the first two lectures in this series (Douglas, 1968; Vane, 1969). However, I have consoled myself with the thought that at least the substance of my lecture may be considered to be relevant to this occasion, since I propose to discuss certain aspects of adrenergic neurotransmission. As you will know, this was a field in which Gaddum and his colleagues were involved in the early demonstrations of the existence and release of noradrenaline from sympathetic nerve terminals and in the development of sensitive assay methods for the catecholamines (Gaddum & Kwiatkowski, 1938, 1939; Gaddum & Goodwin, 1947; Gaddum, Peart & Vogt, 1949; Gaddum & Lembeck, 1949; Gaddum, 1950; Gaddum & Holzbauer, 1957). I shall be especially concerned with the mechanisms which are thought to be responsible for terminating the actions of noradrenaline after its release from adrenergic nerve terminals, and this is also a topic of which Gaddum was well aware more than 30 years ago, as the following quotation from Gaddum & Kwiatkowski (1938) makes clear: "Experiments designed for the demonstration of the liberation of acetylcholine have been greatly facilitated by the use of eserine, which inhibits the hydrolysis of acetylcholine by choline esterase. If a substance could be discovered which would protect adrenaline as eserine protects acetylcholine, experiments with adrenergic nerves might be made much easier."

I hope to show that even now the perfect homologue of eserine for the adrenergic synapse may not have been discovered but that at least some progress has been made along these lines.

Figure 1 summarizes the various mechanisms which may be involved in terminating the pharmacological actions of neurotransmitters after their release from presynaptic nerve terminals. Simple diffusion of the released transmitter away from the synaptic cleft, and its subsequent dilution in surrounding extracellular fluids is in principle one way in which this process could take place. It has been suggested that diffusion is sufficiently rapid to account for the termination of the actions of 5-hydroxytryptamine in molluscan ganglia (Gerschenfeld & Stefani, 1968). A more familiar mechanism involves the metabolic conversion of the released transmitter into pharmacologically inactive metabolites, as in the metabolism of acetylcholine by the enzyme acetylcholinesterase at cholinergic synapses. The third type of inactivation

mechanism is the one which I will emphasize in this lecture, namely the removal of the released transmitter from the synaptic cleft by means of a membrane transport system located adjacent to this region. Such a transport system might in principle be situated in the presynaptic nerve terminal, in the postsynaptic cell, or in adjacent Schwann or glial cells. In any of these locations the end result would be similar: to reduce the concentration of free transmitter in the cleft region. However, in practice it seems that such uptake mechanisms are commonly located in the presynaptic nerve terminal, conferring the added advantage of 'transmitter economy' in the sense that individual molecules of transmitter may undergo several cycles of release, recapture and re-use. The uptake systems associated with adrenergic nerves in the peripheral sympathetic nervous system, are the principal topic of this lecture, but I shall also describe briefly the probable existence of similar uptake systems in neurones in which transmitters other than noradrenaline, such as dopamine, 5-hydroxytryptamine, or gamma-aminobutyric acid (GABA) are involved.

Uptake of catecholamines

Properties of neuronal uptake mechanisms

Sympathetic nerves The ability of postganglionic sympathetic neurones to take up exogenous catecholamines is now well known. The subject has been reviewed recently (Iversen, 1970a, b) and I shall describe only the salient features of this system here. Recent interest in this process stems from the work of Axelrod and his colleagues with tritiated catecholamines about 10 years ago (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961). The radioactively labelled catecholamines were found avidly to be accumulated by sympathetic nerve terminals in various peripheral organs after their intravenous injection in mice and cats.

In many subsequent studies the properties of the uptake mechanism involved have been investigated by exposure of isolated sympathetically innervated organs to labelled catecholamines. My own experiments were performed with the isolated rat heart, perfused through the coronary vasculature by the Langendorff procedure

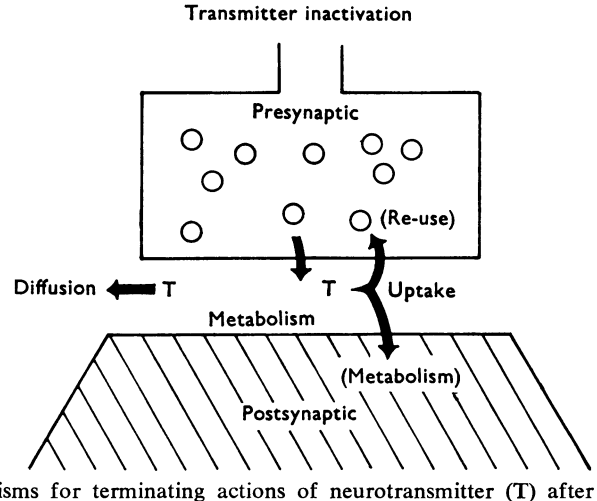

FIG. 1. Mechanisms for terminating actions of neurotransmitter (T) after release from presynaptic nerve terminal Transmitter may be inactivated by diffusion away from synaptic cleft, metabolism in the cleft, uptake into nerve terminal or uptake into postsynaptic or other cell followed by intracellular metabolism.

(Iversen, 1963). The isolated heart rapidly accumulated labelled noradrenaline when perfused with a solution containing a low concentration of (±)-^{3}H-noradrenaline (10 ng/ml). The uptake led to concentrations of ^{3}H-NA in the heart which were about 40 times greater than that in the perfusion medium after perfusion for 20 minutes. This means that the noradrenaline uptake system is capable of clearing the entire extracellular space of the heart in approximately 10 seconds. If one considers that the uptake sites are restricted to the sympathetic nerve terminals in the heart, which occupy only a minor proportion of the tissue volume, this performance is even more remarkable. There is no way of making an accurate estimate of the volume of extracellular space adjacent to the sympathetic terminals, but it seems likely that the uptake system could completely clear noradrenaline from this fluid volume in an interval of time measured in milliseconds rather than seconds.

That the uptake is restricted to sympathetic nerves was confirmed by the finding that uptake of ^{3}H-NA was reduced by more than 95% in rat hearts in which the

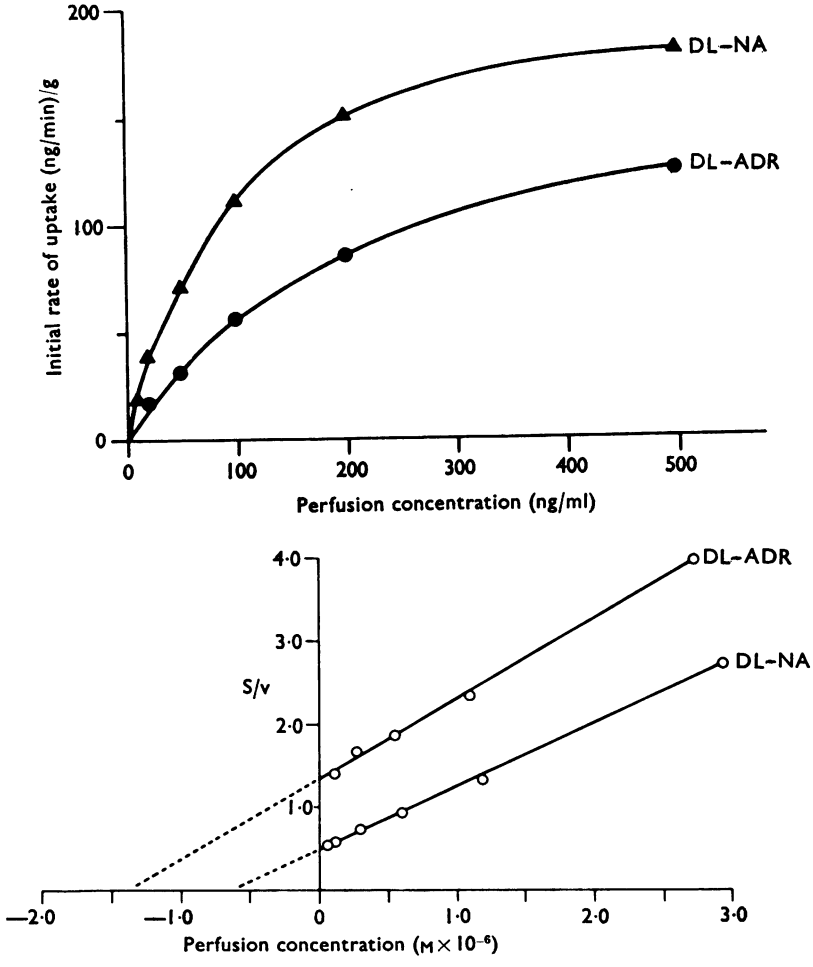

FIG. 2. Kinetic analysis of catecholamine uptake by isolated rat heart. Initial rates of uptake (v) show saturation with increasing perfusion concentrations of amine (S). These results can be fitted to a form of the Michaelis-Menten equation (lower part of figure) from which kinetic constants can be derived. (Iversen, 1967).

sympathetic innervation was lacking through immunosympathectomy at birth (Iversen, Glowinski & Axelrod, 1966). Other experiments established that the uptake of ^{3}H-NA by the isolated rat heart was followed by a firm retention of the accumulated amine, without significant metabolic destruction or wash-out. This retention process involves the transfer of the accumulated amine into intraneuronal storage vesicles. The initial uptake process is mediated by a mechanism which is temperature dependent, requires the presence of sodium ions in the external medium, is inhibited by metabolic poisons and by ouabain, and exhibits saturation kinetics (Fig. 2). The kinetics constants for various substrates tested (Table 1) suggest that the uptake is specific in favour of the naturally occurring (−)-isomer of noradrenaline and in preferring noradrenaline to adrenaline. The uptake process is inhibited by a wide range of sympathomimetic amines structurally related to noradrenaline, and from the results of such experiments information has been obtained on the structure activity relationships required for optimal binding of β-phenylethylamines to the noradrenaline uptake sites on sympathetic nerve terminals (Burgen & Iversen, 1965). Such binding occurs with very high affinity for certain amines such as (−)-metaraminol, which binds with an affinity about 4 times higher than that of the natural substrate (−)-noradrenaline.

Binding of sympathomimetic amines to the uptake sites may simply inhibit the uptake of noradrenaline, without the inhibiting amine itself being transported into the neurone (for example amphetamine) (Thoenen, Hürlimann & Haefely, 1968). Alternatively, many sympathomimetic amines may compete with noradrenaline for binding to the uptake sites and this may be followed by transport of the competing amine into the neurone (for example α-methyl tyramine) (Iversen, 1966). The noradrenaline uptake process is also potently inhibited by other drugs such as cocaine, phenoxybenzamine and various imipramine derivatives. All of these compounds appear to act competitively with noradrenaline, and some have very high affinities for the uptake sites (Callingham, 1967 ; Iversen, 1970a, b).

Uptake of noradrenaline by sympathetic nerves in tissues other than the heart, and in various mammalian species, seems to be mediated by a process very similar to that described in the rat heart, although there are quantitative differences in the kinetic parameters of the process in different species (Iversen, 1970b).

In summary postganglionic sympathetic neurones have the ability actively to accumulate and retain exogenous noradrenaline and certain related β-phenylethylamines. This process is mediated by a mechanism which has the properties of an active transport membrane carrier system, and is probably located in the axonal membrane of these neurones.

Catecholamine-containing neurones in the CNS

Neurones in the mammalian CNS which contain noradrenaline or dopamine are also capable of an uptake of exogenous catecholamines. The kinetics of these

TABLE 1. *Kinetic constants for catecholamine uptake (Uptake$_1$) in rat heart*

Compound	Michaelis Constant −Km (M × 10^{-6})	Max. rate uptake ((nmol/min)/g heart)
DL-noradrenaline	0·67	1·36
L-noradrenaline	0·27	1·18
D-noradrenaline	1·39	1·72
DL-adrenaline	1·40	1·03

From Iversen (1967).

uptake processes have recently been studied in adrenergic nerve terminals (synaptosomes) derived by homogenization of brain areas rich in either noradrenaline (for example hypothalamus) or dopamine (neostriatum) (Colburn, Goodwin, Murphy, Bunney & Davis, 1968 ; Snyder & Coyle, 1968 ; Coyle & Snyder, 1969a, b ; White & Keen, 1970). The results of these studies suggest that the uptake process responsible for catecholamine accumulation by noradrenaline-containing nerve terminals is similar to, and perhaps identical with, the process described in peripheral sympathetic neurones. The kinetic constants and inhibitor specificities of the two processes appear to be closely similar (Tables 1 and 2). On the other hand, dopamine-containing nerve terminals accumulate external catecholamines by a different mechanism. The uptake process in these neurones will accumulate both noradrenaline and dopamine, but has a higher affinity for dopamine, and shows no stereochemical specificity for (+)- and (−)-noradrenaline. Furthermore, the uptake of catecholamine into dopamine neurones is only slightly inhibited by imipramine and its analogues, but is very greatly inhibited by (−)-amphetamine and by certain anti-Parkinsonian drugs such as benztropine, which are not as potent in inhibiting uptake by noradrenaline neurones (Hamberger, 1967 ; Coyle & Snyder, 1969a, b). Clearly much more remains to be learned about the properties of this catecholamine uptake system.

Extraneuronal uptake of catecholamines—Uptake$_2$

In addition to the uptake of catecholamines by neuronal mechanisms, a mediated uptake of exogenous amines also takes place into certain non-neuronal tissues. In the rat heart, for example, a process which for convenience has been termed Uptake$_2$ (Iversen, 1965) transports noradrenaline and other catecholamines into the cardiac muscle cells (Farnebo & Malmfors, 1969 ; Clarke, Jones & Linley, 1969). In the isolated perfused rat heart this process was first detected when very high perfusion concentrations of adrenaline or noradrenaline were used (>1 μg/ml) (Figs. 3 and 4). Under these conditions there was a large accumulation of unchanged catecholamine, which was not firmly retained but was readily washed out of the tissue if perfusion was continued with an amine-free solution (Fig. 4). The mechanism responsible for this accumulation again shows properties consistent with a mediated transport mechanism, but the detailed properties of this uptake are clearly distinguishable from those associated with the neuronal process (Uptake$_1$). Uptake$_2$ is a low affinity system, but with maximum rates of uptake much greater than those associated with Uptake$_1$ (Table 3). Uptake$_2$ is not stereochemically specific, and unlike Uptake$_1$ has a higher affinity for adrenaline than for noradrenaline. Isoprenaline shows an even higher affinity for Uptake$_2$ (Callingham & Burgen, 1966), although this catecholamine does not appear to be accumulated at all by Uptake$_1$.

TABLE 2. *Kinetic constants for uptake of (+)-^{14}C-noradrenaline (NA) and (−)-^{3}H-noradrenaline by synaptosomes in homogenates of regions of rat brain rich in dopamine terminals or NA terminals*

	(+)-NA		(−)-NA	
	Km-μM	Vmax ((nmol/g)/min)	Km-μM	Vmax ((nmol/g)/min)
Dopamine terminals (neostriatum)	1·9	2·8	2·0	2·3
NA terminals (hypothalamus)	0·8	1·1	0·2*	0·6*

*=P<0·01 when compared with values for (+)-NA. (Iversen, unpublished results.)

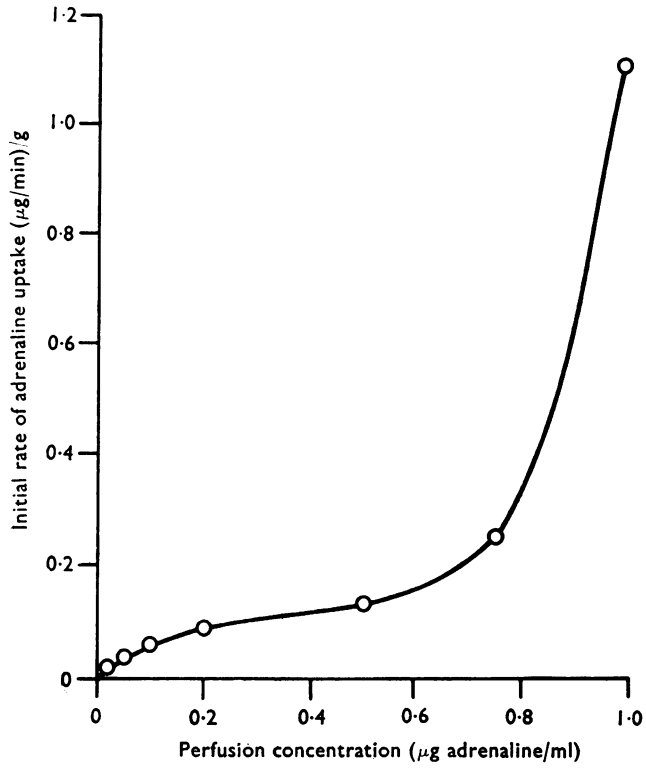

FIG. 3. Initial rate of uptake of adrenaline in isolated rat heart perfused with various concentrations of the catecholamine, illustrating emergence of $Uptake_2$ at high perfusion concentrations (Iversen, 1965).

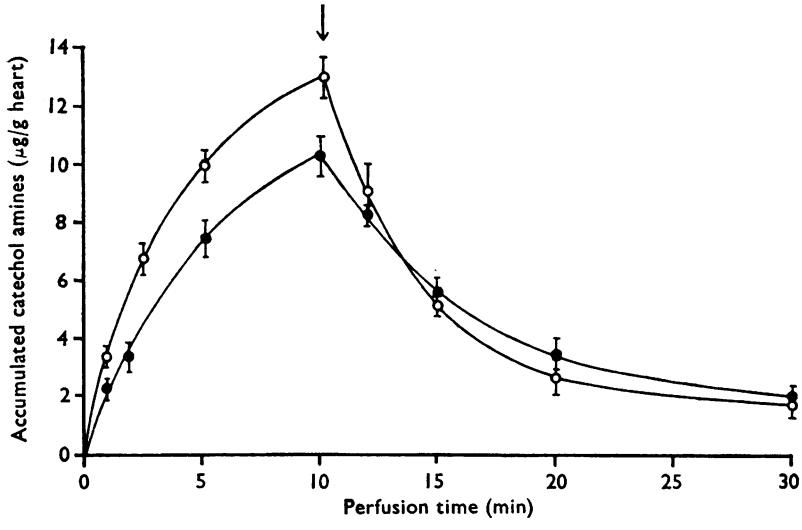

FIG. 4. Uptake of adrenaline by rat heart during perfusion with adrenaline (○——○) or noradrenaline (●——●) (5 μg/ml), is followed by a rapid wash-out of the accumulated catecholamine when perfusion is continued with an amine-free medium (arrow) (Iversen, 1965).

Uptake$_2$ is also inhibited by a different group of drugs from those found to be effective as Uptake$_1$ inhibitors (Burgen & Iversen, 1965). Among the most potent inhibitors of Uptake$_2$ are the methoxylated metabolites normetanephrine and metanephrine and phenoxybenzamine. We have recently found that certain steroids are also particularly powerful inhibitors of Uptake$_2$ (Fig. 5), and most of these substances were not active as inhibitors of Uptake$_1$ (Iversen & Salt, 1970).

It has also become apparent that Uptake$_2$ is not a 'threshold' phenomenon as was originally thought. That is to say, Uptake$_2$ does not abruptly become activated at certain critical concentrations of external catecholamine. Instead it seems likely that Uptake$_2$ operates throughout the range of external catecholamine concentrations, but that at low amine concentrations the accumulated catecholamines are rapidly metabolized by monoamine oxidase and catechol-O-methyl transferase in

TABLE 3. *Kinetic constants for catecholamine Uptake$_2$ in rat heart*

Compound	Km—(M × 10^{-6})	Max. rate uptake ((nmol/min)/g heart)
DL-noradrenaline	252·0	100
DL-adrenaline	51·6	64·4
DL-isoprenaline	23·0	15,5

From Iversen (1965) and Callingham & Burgen (1966).

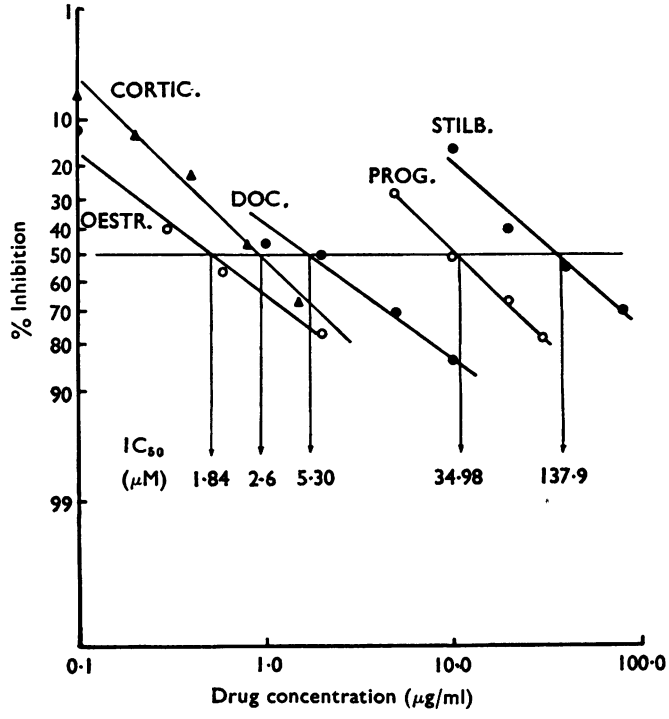

FIG. 5. Determination of Uptake$_2$ IC50 values for steroids in isolated rat heart. Inhibition of Uptake$_2$ (probability scale) by addition of steroids to the perfusion medium is plotted against steroid concentration (log scale) to determine IC50 (concentration producing 50% inhibition). Each point is the mean value for six hearts. OESTR = 17-β-oestradiol; CORTIC = corticosterone; DOC = deoxycorticosterone; PROG = progesterone; STILB = diethylstilboestrol. (Iversen & Salt, 1970).

the cardiac muscle cells. There is thus no accumulation of unchanged catecholamine unless high perfusion concentrations of external catecholamine are used, when the rate of uptake of catecholamine by $Uptake_2$ will exceed the metabolic capacity of the degradative enzymes. $Uptake_2$ can be demonstrated even at relatively low perfusion concentrations of noradrenaline (0·5 μg/ml) if the two degradative enzymes are inhibited (Lightman & Iversen, 1969) (Fig. 6). Assuming that both $Uptake_1$ and $Uptake_2$ do operate at all catecholamine concentrations, it is possible to predict the respective rates of uptake into the rat heart by these two processes from a knowledge of the respective kinetic constants (Fig. 7).

It seems probable that a process similar or identical to $Uptake_2$ also exists in many other tissues innervated by the sympathetic system. The histochemical studies of Gillespie and his colleagues suggest that the extraneuronal uptake of noradrenaline also occurs in many types of vascular smooth muscle, and in other smooth muscle tissues such as vas deferens, spleen and cat nictitating membrane (Avakian & Gillespie, 1968 ; Draskoczy & Trendelenburg, 1970 ; Gillespie, Hamilton & Hosie, 1970 ; Gillespie & Muir, 1970).

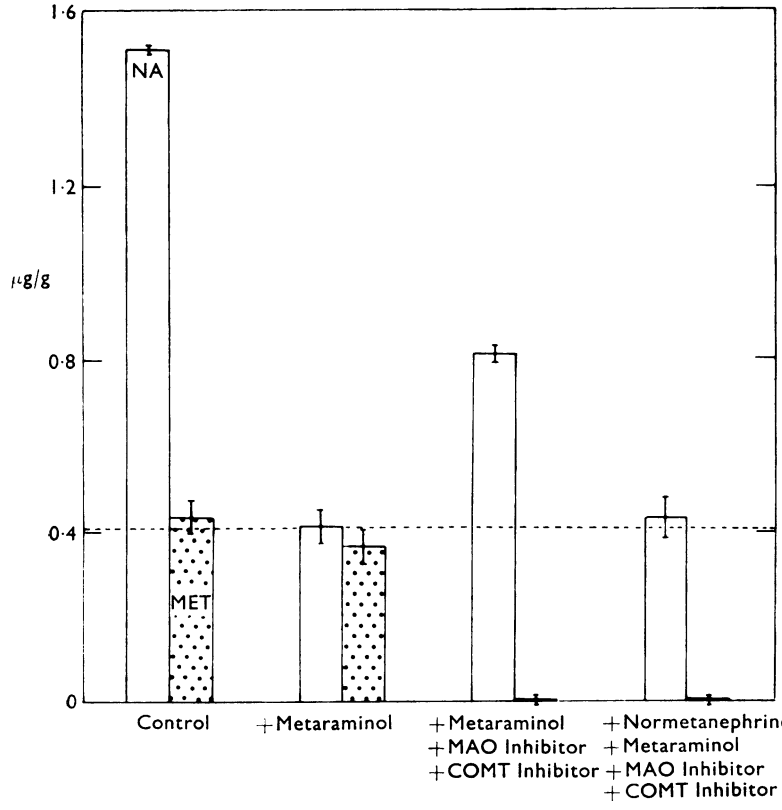

FIG. 6. ^{3}H-noradrenaline (NA) and labelled metabolites (shaded histograms) in the rat heart at the end of 10 min perfusions with NA (0·5 μg/ml), in the presence of metaraminol (0·5 mM), inhibitors of metabolism and normetanephrine (5 μM). Each value is the mean of four–six hearts. Dotted line indicates ^{3}H-noradrenaline content of metaraminol treated group. (Lightman & Iversen, 1969).

Physiological functions of catecholamine uptake processes

The hypothesis that noradrenaline is inactivated at adrenergic nerve terminals principally by a recapture of the released transmitter, mediated by the neuronal uptake process, is quite widely accepted. The arguments in favour of this view have been summarized elsewhere, and I propose to deal with only some selected aspects of this topic (Iversen, 1967 ; Ferry, 1967 ; Iversen, 1970a, b). Figure 8 summarizes the possible fates of noradrenaline after release from adrenergic nerve terminals. The transmitter may be removed from the extracellular space either by an uptake ($Uptake_1$) and return to storage sites in the adrenergic nerve terminal, or it may be taken up ($Uptake_2$) into postsynaptic tissues in which it will be metabolically degraded. It is generally assumed that the neuronal recapture mechanism is the

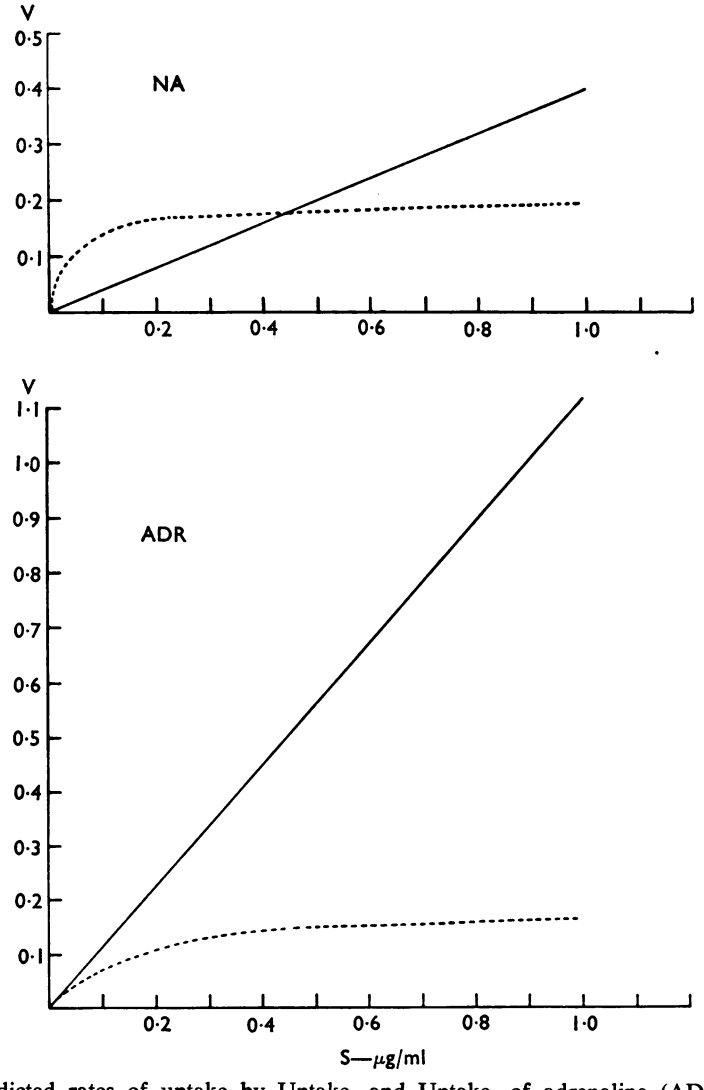

FIG. 7. Predicted rates of uptake by $Uptake_1$ and $Uptake_2$ of adrenaline (ADR) and noradrenaline (NA) by isolated rat heart with various perfusion concentrations of catecholamine. $Uptake_1$ rates as dotted lines, $Uptake_2$ as solid lines. (Lightman & Iversen, 1969).

principal mechanism involved, but it is possible that $Uptake_2$ followed by metabolism might also play some role in the inactivation process.

Perhaps the most important evidence in favour of the view that $Uptake_1$ is the principal inactivation mechanism has come from experiments in which it was shown that the 'overflow' of noradrenaline from isolated organs in response to sympathetic nerve stimulation was greatly increased by drugs known to inhibit the neuronal recapture mechanism (Brown, 1965 ; Thoenen, Hürlimann & Haefely, 1964a, b). Unfortunately, however, the ideal inhibitor of $Uptake_1$ has not yet been discovered. Experiments with drugs such as cocaine and imipramine have yielded equivocal results, probably because these substances interfere with the propagation of nerve impulses in sympathetic nerves if used at concentrations sufficiently high to inhibit the neuronal uptake mechanism. Phenoxybenzamine, on the other hand, is a potent uptake inhibitor without the complications of local anaesthetic activity. This drug consistently produces large increases in the overflow of noradrenaline from stimulated organs (Brown & Gillespie, 1957 ; Kirpekar & Cervoni, 1963 ; Brown, 1965 ; Thoenen *et al.*, 1964a, b). Unfortunately, however, phenoxybenzamine is also a powerful inhibitor of the $Uptake_2$ mechanism.

The recent studies of Langer (1970) suggest that the effects of phenoxybenzamine on the overflow of released noradrenaline may be due, at least in part, to an inhibition of $Uptake_2$. Langer examined the release of unchanged ^{3}H-noradrenaline and labelled metabolites from the isolated cat nictitating membrane in response to electrical stimulation. In response to low frequencies of stimulation there was usually only a small overflow of radioactive materials, and unchanged noradrenaline accounted for a small proportion of this radioactivity. In the presence of phenoxybenzamine, however, there was both a large increase in the overflow of radioactivity,

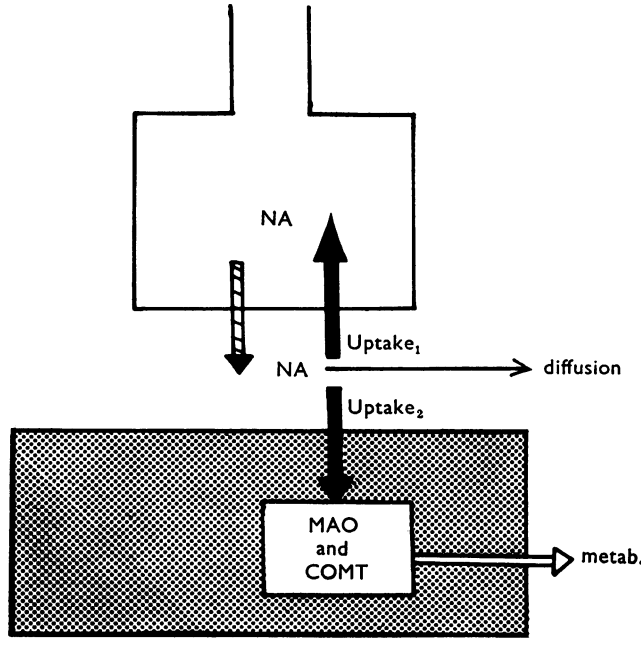

FIG. 8. Possible mechanisms for inactivation of noradrenaline (NA) at adrenergic nerve terminals.

and a marked shift in the pattern of labelled materials in this overflow. In the presence of phenoxybenzamine the overflow was accounted for almost exclusively as unchanged ^{3}H-noradrenaline, and not by noradrenaline metabolites. This effect of phenoxybenzamine in preventing the extraneuronal metabolism of the released ^{3}H-noradrenaline can almost certainly be ascribed to an inhibition of $Uptake_2$ produced by this drug. Nevertheless, phenoxybenzamine (and cocaine) did produce an increase in the total overflow of radioactivity, and this presumably reflects an inhibition of the neuronal recapture mechanism by the drug. On this basis $Uptake_1$ would appear to account for the recapture of about 70–90% of the transmitter released by low frequencies of sympathetic nerve stimulation in the nictitating membrane. The remainder of the released transmitter may be removed by $Uptake_2$ and subsequent extraneuronal metabolism. One would expect the relative quantitative importance of neuronal recapture ($Uptake_1$) versus the $Uptake_2$-and-metabolism mechanism to vary from one tissue to another, since the relative density of $Uptake_1$ and $Uptake_2$ sites will vary according to the density of the sympathetic innervation. It is plausible to suppose, for example, that $Uptake_2$-and-metabolism might be the predominant mechanism for noradrenaline inactivation in tissues such as vascular smooth muscle, in which the density of sympathetic innervation in certain parts of the tissue is very low. Further experimental tests will be needed, however, to determine the precise importance of the $Uptake_2$ mechanism in adrenergic neurotransmission.

It is also possible that $Uptake_2$ plays an important role in the removal and inactivation of circulating catecholamines. Circulating adrenaline, for example, would be expected to be rapidly removed by $Uptake_2$ sites (for which it has a high affinity) in the smooth muscle tissue of the capillary vasculature. The experiments of Axelrod *et al.* (1959) and Whitby *et al.* (1961), however, suggest that the uptake and retention of circulating adrenaline and noradrenaline by sympathetic nerves also play a significant role in the removal of circulating catecholamines. After intravenous injections of small doses, about 35% of the injected dose of ^{3}H-adrenaline and about 55% of the injected dose of ^{3}H-noradrenaline appeared to be taken up and retained by the neuronal mechanism (Iversen & Whitby, 1962). It is not my intention to dwell on this topic, since my predecessor Professor Vane has discussed the inactivation of circulating catecholamines with far greater erudition in the previous Gaddum Lecture (Vane, 1969).

Some pharmacological implications of catecholamine uptake processes

Apart from the role of catecholamine uptake in the physiological disposition of the catecholamines, an understanding of the various uptake systems can also help to elucidate the mode of action of various drugs on adrenergic mechanisms. In general, for example, drugs that inhibit the neuronal uptake of catecholamines will be expected to potentiate and perhaps to prolong the pharmacological effects of the catecholamines. This will be true both for the effects of noradrenaline released endogenously by sympathetic nerve activity and for the effects of exogenously applied catecholamines. There seems little doubt that the potentiation of the actions of noradrenaline and other adrenergic agonists by cocaine can be explained in this way. Cocaine potentiates the effects of noradrenaline to a greater extent than those of adrenaline (which is less effectively removed by $Uptake_1$) ; the potentiating effects of cocaine are, furthermore, greatest in tissues with a high density of neuronal

uptake sites such as the cat nictitating membrane, and least in tissues with only a sparse sympathetic innervation, such as aortic smooth muscle (Trendelenburg, Draskoczy & Pluchino, 1969). Cocaine also fails to potentiate the actions of catecholamines in tissues which lack a normal sympathetic innervation (Trendelenburg, 1966). Not all inhibitors of $Uptake_1$ cause a potentiation of the effects of catecholamines, since such drugs will often have other actions which obscure such an effect. For example, chlorpromazine is a potent uptake inhibitor, but is also active as an adrenergic α-adrenoceptor antagonist (Callingham, 1967).

Furthermore, the effects of adrenergic agonists other than (−)-noradrenaline will not always be potentiated by uptake inhibition. Such a potentiation will only occur if the following conditions are fulfilled: (1) the agonist is a substrate for uptake, with an affinity constant=K, (2) the agonist elicits a pharmacological response in a dose range at which uptake remains unsaturated; that is $ED50 \leqslant K$. Under these conditions the concentration of agonist in the extracellular space adjacent to the receptor sites will be reduced to a value lower than that applied to the external medium, because of the continuous removal of the agonist from the biophase by uptake (Fig. 9). In general, uptake will, therefore, only tend to be an important factor in limiting the actions of potent agonists (Iversen, 1970b). These considerations are discussed at greater length in the recent paper of Langer & Trendelenburg (1969). The phenomenon is well illustrated by the finding that the more potent (−)-isomers of noradrenaline and adrenaline are markedly potentiated by cocaine, whereas the less potent (+)-isomers are much less affected by the drug (Draskoczy & Trendelenburg, 1968).

There have been rather few studies of the effects of $Uptake_2$ inhibitors on adrenergic responses, but it is known that in some cases these compounds may also

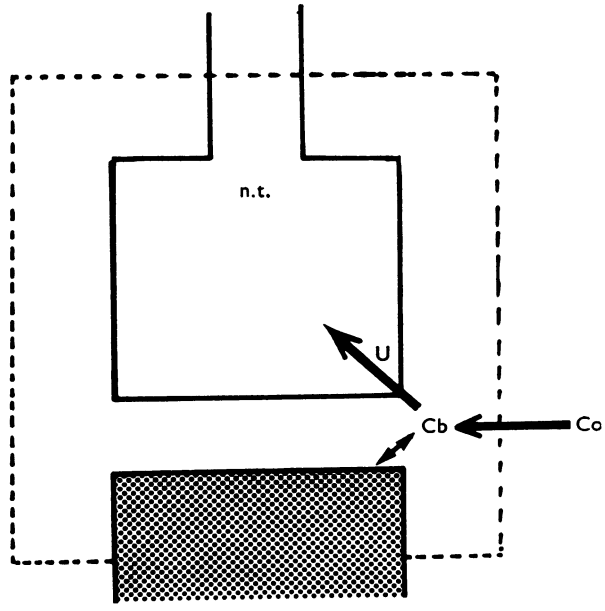

FIG. 9. Role of $Uptake_1$ (U) in limiting the biophase (dotted line) concentration of adrenergic agonists. Co=concentration of agonist in external medium, Cb=agonist concentration in biophase.

potentiate the actions of catecholamines. Thus, in tracheal smooth muscle the effects of isoprenaline were found to be potentiated by metanephrine (Foster, 1969), and the effects of various adrenergic agonists on aortic smooth muscle were potentiated by the steroids which were subsequently found to be active as $Uptake_2$ inhibitors (Kalsner, 1969a, b). It is possible that the potentiation of catecholamine actions by steroids recorded in many biological test systems (Schayer, 1964) may be related to their action on $Uptake_2$.

Another phenomenon of pharmacological interest in which the neuronal uptake of catecholamines has been implicated is the development of supersensitivity to catecholamines in sympathetically denervated organs. It is now well established that a major factor underlying such denervation sensitivity is the loss of neuronal uptake sites which accompanies the degeneration of the terminal sympathetic innervation (Hertting, Suko, Widhalms & Harbich, 1967; Langer, Draskoczy & Trendelenburg, 1967).

In addition brief mention should be made of the role of the neuronal uptake process in the entry and subsequent retention of amines related to noradrenaline as 'false transmitters' in adrenergic neurones (Muscholl, 1966; Kopin, 1968; Thoenen, 1969). Two compounds which fall within this general category have been found to have additional properties which render them uniquely useful as research tools: 5-hydroxydopamine and 6-hydroxydopamine. The former compound is taken up and retained by peripheral adrenergic nerves, and has the valuable property of rendering the intraneuronal catecholamines storage vesicles intensely osmiophilic, thus facilitating the identification of adrenergic terminals at the electron microscope level (Tranzer & Thoenen, 1967).

The latter compound, 6-hydroxydopamine, is also accumulated by adrenergic neurones, both in the periphery and in the CNS, but has the remarkable property of causing cellular damage leading to a complete degeneration of adrenergic nerve terminals (Thoenen & Tranzer, 1968; Jonsson & Sachs, 1970; Uretsky & Iversen, 1970).

The ability to label adrenergic nerve terminals selectively by exposing tissues to radioactively labelled catecholamines is another useful research method which stems from the existence of specific uptake mechanisms in adrenergic neurones for these substances. Such approaches have been extremely valuable for studies of the metabolism, release, storage, rate of turnover and subcellular distribution of the catecholamines in adrenergic neurones both in the periphery and in the CNS (Iversen, 1967; Glowinski & Baldessarini, 1966; Axelrod & Kopin, 1969).

Uptake of other transmitter substances

GABA

Gamma-aminobutyric acid (GABA) is the inhibitory neurotransmitter released at crustacean neuromuscular junctions (Otsuka, Iversen, Hall & Kravitz, 1966; Potter, 1968). There is also a rapidly growing body of evidence which favours the view that GABA plays a similar role as an inhibitory neurotransmitter at certain synapses in the mammalian CNS, notably in the cerebellum and cerebral cortex (Curtis & Watkins, 1965; Curtis & Crawford, 1969; Hebb, 1970; Krnjevic, 1970).

Although a catabolic enzyme, GABA-glutamate transaminase exists in mammalian brain, it seems unlikely that enzymic breakdown can account for the in-

activation of GABA after its release from inhibitory nerve terminals either in the crustacean or mammalian systems. The transaminase enzyme is localized in mitochondria and furthermore requires the presence of both pyridoxal phosphate and α-ketoglutarate, making it unlikely that extracellular GABA is susceptible to attack by this enzyme. Inhibitors of the transaminase are also ineffective in potentiating or prolonging either inhibitory synaptic transmission or the effects of exogenously applied GABA at inhibitory synapses (Curtis & Watkins, 1965). Instead, it seems more probable that free GABA is inactivated by an uptake process which removes it from its extracellular site of action. In both crustacean nerve-muscle systems and mammalian brain an uptake of exogenous GABA has been observed (Sisken & Roberts, 1964 ; Blasberg & Lajtha, 1965 ; Weinstein, Varon, Muhleman & Roberts, 1965 ; Iversen & Kravitz, 1968 ; Iversen & Neal, 1968 ; Strasberg & Elliott, 1967). The uptake system which we are most interested in is that present in rat cerebral cortex (Iversen & Neal, 1968). This is capable of a very rapid concentration of extracellular tritium labelled GABA (Fig. 10), leading to tissue to medium ratios as high as 100 to 1 when small slices of cortex are incubated with the radioactive amino-acid in an artificial saline medium. This rate of uptake is even more rapid than that of labelled noradrenaline by the isolated rat heart ; it corresponds to a complete clearance of GABA from the extracellular space of the cortical slices in

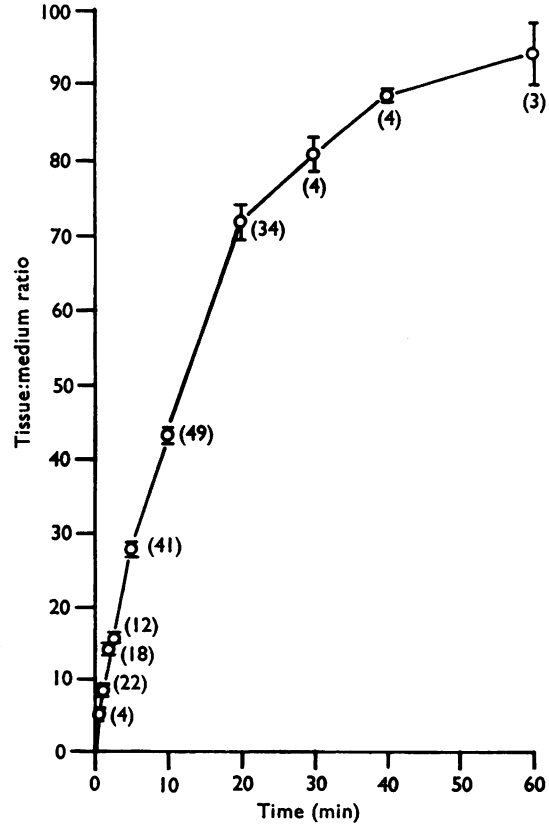

FIG. 10. Uptake of ^{3}H-GABA by slices of rat cerebral cortex incubated with ^{3}H-GABA (0·05 μM) *in vitro*. Results are expressed as tissue to medium ratio and are means ± SEM for No. observations indicated in brackets. (Iversen & Neal, 1968).

less than 2 seconds. Uptake of GABA is similar to that of noradrenaline in that it appears to be mediated by an active transport system which is temperature dependent, saturable, sodium dependent and highly specific. The Km value for GABA uptake is 22·0 μM, and the maximum rate of uptake=(0·115 μmol/g cortex)/minute. This means that the GABA uptake process is a particularly high affinity uptake system, when compared with those which exist in brain and other tissues for the transport of amino-acids ; such systems have Km values generally in the range 0·5–5·0 mM (Blasberg & Lajtha, 1965).

Unfortunately, very few potent inhibitors of the GABA uptake process are known. Table 4 lists the small number of active compounds which we have encountered in screening various potential inhibitors of this system. Until satisfactory inhibitors have been discovered it will be difficult to test the hypothesis that the GABA uptake process is involved in terminating the actions of released GABA in the mammalian brain. Very recently we have obtained additional information which suggests another similarity between the GABA uptake process and the noradrenaline uptake system of adrenergic nerves. Dr. F. Bloom and I found that by electron microscope autoradiography it was possible to localize the cellular sites of ^{3}H-GABA uptake in slices of rat cerebral cortex. These appear to be predominantly associated with a certain population of nerve terminals in the cortex, accounting for approximately one-third of the total nerve terminal population. This finding adds further support to previous findings from subcellular distribution studies that a substantial proportion of the labelled amino-acid was accumulated by nerve terminals in the cortex, and that these terminals appeared to be the same as those which contain both the endogenous amino-acid and the enzyme involved in GABA biosynthesis, glutamate decarboxylase (Neal and Iversen, 1969). After uptake by such nerve terminals, labelled GABA can subsequently be released by appropriate electrical stimulation (Mitchell & Srinivasan, 1969), suggesting that this will prove to be a valuable tool for investigating the properties of GABA inhibitory terminals in the brain.

Glycine

Although it seems probable that GABA plays an important role as an inhibitory neurotransmitter in the mammalian CNS, it is unlikely to be the only inhibitory transmitter. A substantial body of evidence suggests that the amino-acid glycine is also an inhibitory neurotransmitter, particularly in the mammalian spinal cord (Aprison & Werman, 1965 ; Curtis, Hösli & Johnston, 1968 ; Wermann, Davidoff & Aprison, 1967 ; Curtis, 1969 ; Hebb, 1970). It is, therefore, interesting to find that a specific uptake process for labelled glycine exists in spinal cord (Neal & Pickles, 1968). We have recently confirmed these findings, and have compared the uptake

TABLE 4. *Inhibitors of ^{3}H-GABA uptake by slices of rat cerebral cortex*

	μM GABA—Km=22·0 IC50 or Ki
α-Fluoro-GABA	20·0
L-2,4-Diaminobutyrate	30·0
DL-β-Hydroxy-GABA	170·0
β-Hydrazinopropionate	200·0
β-Guanidinopropionate	220·0

Iversen (unpublished results).

of labelled glycine by slices of rat cerebral cortex and spinal cord (Fig. 11). (Johnston & Iversen, unpublished results).

Our results confirm the existence in rat spinal cord of an uptake system for glycine with a relatively high affinity for the amino-acid (Km=33 μM). On the other hand, the uptake of glycine by cerebral cortex was less rapid than in spinal cord and was mediated by a relatively low affinity system (Km=250 μM). The latter process probably corresponds to the uptake process for neutral amino-acids which is present in most parts of the brain (Blasberg & Lajtha, 1965). The glycine uptake process in spinal cord, however, appears to be unique and may be related to the role of this amino-acid in synaptic transmission in this region of the CNS.

5-Hydroxytryptamine

To complete this catalogue of uptake mechanisms I will briefly describe those which involve 5-hydroxytryptamine (5-HT). There is extensive evidence in favour of an excitatory transmitter role for 5-HT in the cardiac innervation of molluscs (Welsh, 1957). The exquisite sensitivity of the molluscan heart to 5-HT was studied by Gaddum, and formed the basis of a sensitive bioassay method for this amine (Gaddum & Paasonen, 1955). Recently it has been found that the molluscan heart (*Aplysia*) rapidly accumulated labelled 5-HT, and that the labelled amine could be released by stimulation of the cardiac nerves (Chase, Breese, Carpenter, Schanberg & Kopin, 1968). However, no detailed information is available on the properties of the uptake system for 5-HT which presumably exists in the cardiac 5-HT nerves.

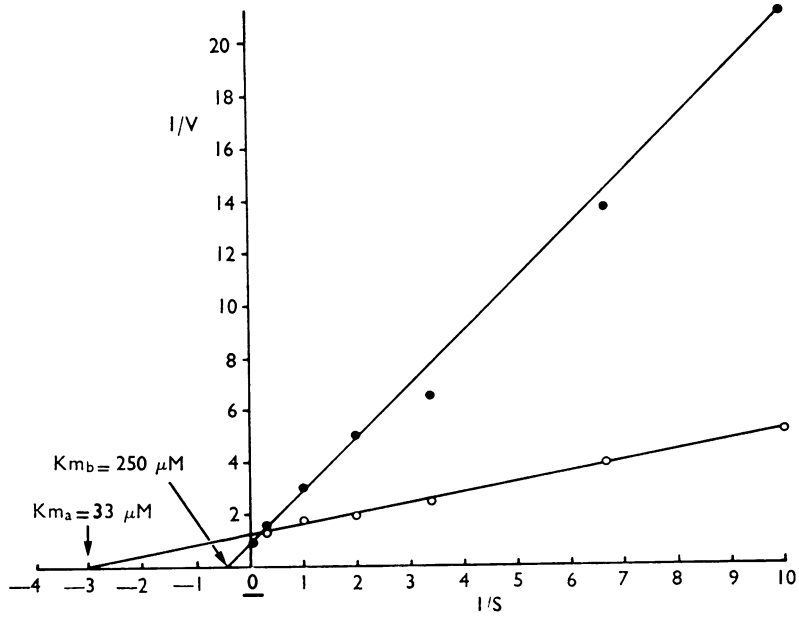

FIG. 11. Kinetic analysis of ^{14}C-glycine uptake in slices of rat spinal cord (○——○) and cerebral cortex (●——●). Initial rates of uptake (v) at various external concentrations of glycine (S= $\times 10^{-4}$M) obey Michaelis-Menten kinetics, and results indicate that uptake in spinal cord occurs with higher affinity (Km$_a$) than in cortex (Km$_b$). (Johnston & Iversen, unpublished results.)

In the mammalian brain certain neurones contain high concentrations of 5-HT, and brain slices or synaptosome preparations are capable of an active uptake when incubated *in vitro* (Ross & Renyi, 1967 ; Blackburn, French & Merrills, 1967). This uptake process has a very high affinity for 5-HT (Km approx. 0·5 μM), and is potently inhibited by imipramine and by certain related compounds, such as 3-chloro-imipramine (Shaskan & Snyder, 1970). The 5-HT uptake process is clearly distinguished by its kinetic properties and inhibitor sensitivity from the processes responsible for the uptake of either dopamine or noradrenaline (Blackburn *et al.*, 1967 ; Shaskan & Snyder, 1970). However, 5-HT is also taken up by both the dopamine and the noradrenaline uptake processes in the brain, with a moderately high affinity. The specific uptake of 5-HT is, therefore, difficult to study in normal brain tissue, since an uptake of 5-HT occurs also into adrenergic structures. After treatment of rats with intraventricular injections of 6-hydroxydopamine, however, the uptake of 5-HT by adrenergic terminals is virtually abolished, leaving only the specific uptake of the amine, which presumably occurs in 5-HT containing nerve terminals (Iversen, 1970a).

The uptake and storage of 5-HT by blood platelets have been studied far more extensively than the neuronal uptake systems for this amine. The platelet uptake of 5-HT involves an active transport mechanism which has a high affinity for 5-HT (Km=0·3 μM), is sodium-dependent, and is inhibited by imipramine and chloro-imipramine (Born & Gillson, 1959 ; Stacey, 1961 ; Sneddon, 1969 ; Todrick & Tait, 1969). Furthermore, the uptake of 5-HT in blood platelets is followed by an intracellular storage of the accumulated amine in specialized storage vesicles, by a reserpine sensitive process (Pletscher, 1968). The uptake and storage of the amine by blood platelets thus bear many remarkable resemblances to the system thought to operate in 5-HT containing neurones.

Conclusion

The existence of a variety of different transport systems in association with different neurotransmitter substances is so striking that one is tempted to speculate that the association of a high affinity uptake mechanism with each type of chemically transmitting synapse may prove to be a general rule. Cholinergic synapse appear to be exceptions to such a rule, although even here there is an uptake process for choline, and possibly one for acetylcholine (Marchbanks, 1968 ; Schuberth & Sundwall, 1967 ; Liang & Quastel, 1969). The uptake of noradrenaline by sympathetic nerve terminals is the only uptake system for which there is direct evidence of a transmitter inactivation role. The possible importance of the other uptake mechanisms in the inactivation of dopamine, GABA, glycine and 5-HT remains to be established, although *a priori* such a functional role would seem probable. In most cases suitable inhibitors of the various uptake processes are not available, although such compounds may in the future prove to be valuable pharmacological tools for the pharmacological manipulation of synaptic transmission at various synapses.

It is clear that our present knowledge of transmitter uptake systems is only rudimentary ; the importance of such mechanisms, both physiologically and pharmacologically, however, seems beyond doubt. 'Our extensive knowledge of transmission by acetylcholine in skeletal muscle and ganglionic synapses has tended to load our minds with preconceptions and inapplicable models' (Brown, 1965).

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(*Received December* 18, 1970)